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ION EFFECTS IN 'ANAEROBIC DIGESTION'

Perry L. McCarty, et al

Stanford University
Stanford, California

March 1964

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PHS Research Grant WP-482, Division of Water Supply
and Pollution Control, Public Health Service
January 1960 to December 1963

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Perry L. McCarty, Project Director
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PHS Research Grant WP-482 from the Division of Water Supply and Pollution Control, Public Health Service, for the period from January 1960 to December 1963.

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ION EFFECTS IN ANAEROBIC DIGESTION

PHS Research Grant WP-482

SUMMARY

This report represents results of a research investigation on the effects of inorganic and organic ions on the anaerobic waste treatment process which was begun in January, 1960, in the Department of Civil Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. At that time the project had the PHS Research Grant No. WP-191. In September, 1962, the principal investigator transferred to the Department of Civil Engineering, Stanford University, Stanford, California, and requested permission to have the grant transferred also. This request was granted and the investigation was completed in December, 1963, under the new PHS Research Grant No. WP-482, which was given at the time of transfer.

The anaerobic treatment process has proven to be an efficient method for the treatment of many organic wastes. It has been used successfully for the treatment of concentrated sludges from municipal waste treatment, as well as both concentrated and dilute waste from industrial processes. The anaerobic treatment process has several advantages over aerobic treatment, one being that the waste is converted primarily to methane gas, a useful end product. Another advantage is that under anaerobic conditions, only a small portion of the organic matter is converted to biological cells, because of the high energy loss in the methane gas produced. Because of this, more complete degradation to gaseous end products is accomplished and much smaller quantities of biological cells are produced for ultimate disposal. In addition, the quantities of nutrients required for biological growth are proportionately less. In spite of these advantages, anaerobic treatment has not been used as widely as it could be. One important reason for this is related to the lack of understanding of the complex effects of high concentrations of inorganic materials normally present in industrial and municipal wastes.

Heavy metals and other materials which may gain access to anaerobic digestion tanks have frequently been blamed for digester failures. Because of the complexity of the process and lack of understanding of the effects of various ions, it has usually been quite difficult to place blame for digester upsets with any real assurance. For this reason, this over-all study was directed toward an understanding of the nature of ion stimulation, inhibition and toxicity, the limits of various ions and combinations of ions which can be tolerated in anaerobic treatment, and methods for ion toxicity control.

The initial studies were concerned with the effects of various cations, as these are normally more toxic than anions to biological processes. The first studies were concerned with cations of the alkali metals, sodium and potassium, the alkaline-earth metals, calcium and magnesium, as well as ammonium. These cations are commonly present in high concentrations in sewage sludges and industrial wastes. Another property of these ions is that they are all relatively soluble under conditions of anaerobic waste treatment. These ions were studied using well defined acetic acid wastes so that the complex ion interaction which results could be better understood. To determine such effects, closely controlled conditions were required.

Each ion was found to have some optimum concentration at which treatment efficiency was best. Concentrations either lower or higher than the optimum levels tended to reduce the efficiency of the process. At higher than optimum concentrations, the rate of treatment and efficiency of the process slowly decreased to a point at which higher cation concentrations became fairly inhibitory to successful operation. It was found that the optimum concentration for each ion was 0.01 M for the monovalent ions sodium, potassium, and ammonium, and 0.005 M for the divalent ions magnesium and calcium. On a molar basis, the order of increasing inhibition of the cations studied when in excess of the optimum concentrations was sodium, ammonium, potassium, calcium and magnesium.

When combinations of these ions were studied, it was found that an optimum concentration of one ion was frequently able to alleviate an inhibitory concentration of another ion. This is called antagonism. Certain ion pairs were also found to be synergistic, that is, high concentrations of one ion tended to increase the inhibition resulting from another ion.

The inhibition caused by excessive concentration of any one of the ions could be antagonized by the addition of the optimum concentration of at least one of the other four cations. Also, maximum antagonism of an inhibitory cation was obtained by the addition of optimum concentrations of several, rather than only one of the other cations.

The results of this study have indicated both optimum concentrations and upper limits of the individual ions which can be tolerated in anaerobic treatment. The study has also indicated which ions can relieve the inhibition caused by other ions and the optimum concentrations of these antagonistic ions which should be used. A method has thus been presented by which the potential inhibition caused by these common ions present in any industrial waste may be evaluated, and how this inhibition might be minimized.

The next series of studies was concerned with the toxicity of the heavy metals, copper, zinc, nickel and iron. It was found that copper, zinc, and nickel, were very toxic to the anaerobic treatment process, while iron had little, if any, effect. It was also found that the toxicity caused by the heavy metals could be completely relieved by precipitating them with sulfides. The significant concentrations of sulfides produced in anaerobic treatment from the degradation of proteins and reduction of sulfates appears to explain the relatively high concentrations of heavy metals often found present and tolerated in anaerobic treatment. It was also found that the concentration of heavy metals which can be tolerated is directly related to the quantity of sulfides present for precipitation. Also, the sum total concentration of heavy metals, rather than that of each heavy metal alone, must be evaluated along with the availability of sulfides for their precipitation in determining heavy metal toxicity. This study has shown that the addition of sulfides

or sulfide precursors to anaerobic treatment processes to relieve heavy metal toxicity is a sound and adequate control procedure. The addition of sulfate salts which acted as sulfide precursors in this study allowed the presence of much higher than 10 percent of each heavy metal on a dried volatile solids basis without inhibiting the digestion process.

Although sulfides can relieve heavy metal toxicity, the addition of sulfides must be done with care, as sulfides are not only corrosive, but are also quite toxic themselves at relatively low concentration. The effect of sulfides on the anaerobic treatment of sewage sludge was evaluated and it was found that a soluble sulfide concentration of up to 200 mg/l could be tolerated, if fed on a daily basis. However, higher concentrations within the digester were detrimental. Because hydrogen sulfide is a gas much of the soluble sulfide formed in anaerobic treatment is lost with the daily digestion gas produced. Because of this loss, much higher concentration that 200 mg/l of sulfides or sulfide precursors can be present in the raw waste. At the loadings used in this study, approximately 75 percent of the sulfides were lost with the gas produced. Thus, an equivalent of 800 mg/l of sulfides in the incoming waste could be tolerated in anaerobic treatment, as the concentration in solution in the digester never exceeded the upper 200 mg/l value. It was also found that precipitation of sulfides with heavy metals rendered them non-toxic. Thus, higher total concentrations of sulfides than 200 mg/l, can be tolerated if they are present as insoluble metal precipitates. Iron salts were found to be particularly suitable for such precipitation, since iron is a relatively non-toxic metal itself.

The final study covered by this investigation was concerned with the effect of high concentrations of individual volatile acids on the anaerobic treatment process. Here, a series of digesters were fed sewage sludge daily and when operation was proceeding normally, slug addition of individual neutralized volatile acids were added. It was found that sudden additions of either acetic or butyric acid to concentrations as high as 8,000 mg/l were found to be stimulatory, rather than inhibitory to the anaerobic treatment process. However, a rapid increase in propionic acid concentration did initially inhibit gas production somewhat. In all cases, this inhibitory effect was overcome by a short period of acclimation. This was true even with propionic acid concentrations as high as 8,000 mg/l. Additional studies indicated that propionic acid affected mainly the acid producing organisms, rather than the methane bacteria themselves. Since such high concentrations of propionic acid would be seldom encountered in practice, it was concluded that the effect of propionic acid was relatively minor and not excessively detrimental to the process. It was also concluded that volatile acids are the result, and not the cause, of failure of the anaerobic treatment process.

Throughout this study the principle of precipitation as a method of controlling or relieving ion toxicity was frequently observed. Thus, the toxicity of any organic or inorganic material can be controlled if a concentration of that material below the toxic level can be achieved by precipitation. This was found true for the heavy metals, for sulfides, for long-chain fatty acids, and for calcium.

One significant finding noted throughout the heavy metal and sulfide toxicity studies, was that gas production was the initial parameter affected at inhibitory concentrations of these materials. In almost all cases, the volatile acids rose to significant concentrations only after gas production had been reduced to low proportions. It appeared these materials initially affected the acid forming bacteria, rather than the methane bacteria as commonly expected. Thus, the general applicability of the volatile acid test as an indicator of the onset of inhibition appears questionable. Change in gas composition was also found a poor indicator. It thus appears that to adequately control the anaerobic treatment process, all the normal digestion parameters must be considered, rather than relying on one parameter alone.

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ION EFFECTS IN ANAEROBIC DIGESTION

I. INTRODUCTION

The ever mounting demand of our civilization for clean water has, in the last twenty years, stimulated increased research on the fundamental principles of waste-water treatment. The greatest attention has been given to biological waste treatment systems. One guiding principle which has developed is that for maximum efficiency of such a treatment system, the environment must be adjusted to favor the growth of the microorganisms responsible for treatment of waste.

Among the environmental factors which exert a major control over all forms of biological life is the ionic constitution of the medium. Biologists and physiologists have studied these ionic phenomena for many years. Although much valuable information has been obtained, no general theory which can account for all the effects observed has been elucidated.

In the design of biological waste disposal systems, the sanitary engineer has taken little cognizance of ion effects because:

(1) The vast majority of wastes treated have been low in salt concentrations.

(2) Ion effects were masked by other more easily recognizable environmental factors.

Among the biological treatment systems, anaerobic digestion holds the greatest promise for the future. This process has the ability to handle a vast variety of wastes at both high and low loadings at very high efficiencies. The "methane bacteria" play the key role in the successful operation of this process. These organisms have the unique ability to ferment alcohols and fatty acids, which are the primary end products of all other anaerobic metabolism, to carbon dioxide and methane.

Unfortunately, of all the microorganisms associated with waste disposal systems, the methane bacteria are by far the most sensitive to any slight change in environmental conditions. To date, little is known about the various environmental factors which will promote optimum proliferation of the methane bacteria.

Cognizance of the ionic environment and its effects is of prime importance in the anaerobic digestion process because:

(1) The methane bacteria grow well only in the narrow pH range 6.5 to 7.5. Consequently, a high buffer must be maintained in the system.

(2) The recent trends toward prethickening of sewage sludge, and reduction of detention time require a faster rate of stabilization in a medium of higher ionic concentration.

(3) Many industrial wastes, particularly amenable to treatment by anaerobic digestion, contain high concentrations of various ions.

(4) Recent research has indicated that ions exert a toxic effect on methane bacteria at lower concentrations than for any other group of bacteria associated with waste disposal systems.

Many ions are present in high concentrations in wastes which can be treated anaerobically. Sodium, potassium, ammonium, calcium, and magnesium are the primary cations normally present, and the effect of these ions, individually and in combination should be understood for effective process design and control. In addition, heavy metals such as copper, zinc, nickel and iron can be quite toxic to biological life and frequently are present in industrial as well as municipal wastes. Information on the toxic levels of these metals is limited and more basic information is desirable to allow evaluation of the toxic potential of wastes as well as to furnish a possible basis for control. Sulfide ions formed from the reduction of sulfates, are quite common and are known to be toxic. Also, some organic compounds, such as the lower fatty acids have been suspected at times to be inhibitory to the anaerobic treatment process.

The purpose of this research investigation is the evaluation of the effects of the above ions on anaerobic biological waste treatment. For convenience, the study has been divided into four phases: (1) Ion Inhibition and Stimulation, covering the effects of the major cations found in wastes, (2) Sulfide Effects, (3) Heavy Metal Effects, and (4) Organic Volatile Acid Effects.

II. ION INHIBITION AND STIMULATION

A. Previous Studies

Prior to a discussion of the previous research into cation effects in anaerobic waste treatment, a review of the basic findings of investigators in the field of pure biology will be given.

Extensive investigations by biologists into the effects of cations on the physiological processes of various organisms have been conducted for the last century. Representative of these are the investigations of Loeb (1) on sea animals and muscle tissue, Osterhaut (2) on plants, and Brooks (3) on bacteria. Falk (4) presented an excellent review of the investigations prior to 1923. These early investigators found that although the quantitative effects of cations varied with the cation and the organisms, the qualitative effects showed no variation. Three basic effects were observed in all cases: toxicity, stimulation, and antagonism.

The first of these phenomena observed was that of toxicity. It was found that all cations could produce a toxic effect in any organism but that the relative toxicity of the cations varied. In general, toxicity increased with the valence and the atomic weight of the cation. Early investigations showed that the toxicity of a cation varied with the presence of other cations in the medium. This ability of one cation to decrease the toxicity produced by another cation was termed antagonism. Antagonistic relationships between many cation pairs were reported (4). For example, Loeb (1) reported antagonisms between thirteen different cation pairs. The most general antagonism reported was that between toxic concentrations of monovalent cations and low concentrations of divalent cations.

The stimulatory effects of cations at concentrations below those where toxicity was produced was also recognized by these early investigators. Brooks (3) reported that peak stimulation by sodium, potassium, calcium and magnesium was achieved at concentrations approximately half those at which toxicity started. A major discovery in this area was made by Hotchkiss (5) who found that very low concentrations of extremely toxic cations such as mercury and lead would produce a stimulatory effect.

Recent research has concentrated on the antagonistic relationships between cations. The early investigators such as Osterhout (2) and Loeb (1) found that antagonism between monovalent and divalent cations was observed only when the cations were present in definite concentration ratios independent of the total concentration. Winslow and Haywood (6) and Winslow and Dolloff (7), however, later reported that, based on their studies on E. coli the concentration ratio was not the controlling factor. They felt that the total effective concentration of all cations present controlled antagonistic effects. Ingram (8) supported the view of these latter investigations. The recent work of MacLeod and Snell (9) on antagonism between monovalent cations has brought forth the ratio principal again. They found that peak antagonism of sodium and ammonium toxicity by potassium was achieved at certain ratios of sodium to potassium and ammonium to potassium regardless of the total ionic concentration.

Perhaps the most important finding of recent studies of cation effects is that cations play a nutritional role in the metabolism of all organisms. They possess a nutritional function by virtue of the fact that many cations serve as metallic activators for a wide variety of enzymes. Dixon and Webb (10) presented a complete list of all known enzymes which require cations as metallic activators.

Despite the vast magnitude of research into the basic nature of cation effects, no single theory has been proposed which can explain all the phenomena observed. At the present time, the most popular theories all postulate some sort of interaction between cations and enzymes. Such a combination is postulated to produce stimulation when the correct metallic activator unites with the enzyme while toxicity results when the enzyme unites with an improper cation. Antagonism is explained as some sort of competition between the functional and non-functional cation for the enzyme surface.

Previous work on cation effects in anaerobic waste treatment has been meager compared to the work performed by the biologists. Indeed, very few studies have been carried out with the specific purpose of determining cation effects. Rather much of the information available has resulted from studies in which cation effects were, so to speak, an afterthought.

Rudolphs (11) found that concentrations of sodium chloride up to 2 g/l stimulated digestion of domestic sewage sludge. Significant retardation of the process was observed at 10 g/l, with methane production affected to a greater extent than volatile matter destruction. Rudolphs and Zeller (12) investigated the effects of 1000 mg/l of various sulfates on the digestion of sewage sludge. Heavy metals were found more toxic than light metal ions. Again, the methane formers were affected to a greater extent than the acid formers.

Fair and Carlson (13) found that the use of calcium carbonate and lime for pH control could markedly speed up batch digestion of sewage sludge. Sodium carbonate and sodium hydroxide, however, were toxic. Cassel and Sawyer (14) reported similar results. Calcium hydroxide and magnesium hydroxide additions for pH control speeded up digestion, while the use of ammonium hydroxide was detrimental.

Snell (15) and Albertson (16) both reported a relationship between pH and the concentration at which ammonium ion produced toxicity to methane organisms. Buswell et al (17) found that in the digestion of milk wastes the process was retarded when the ammonia nitrogen concentration reached the range (1200 - 2500 mg/l). This was the same toxic range as that observed by Albertson (16) for the digestion of sewage sludge. McCarty and McKinney (18) also observed a relationship between pH and ammonium toxicity. This relationship was attributed to the shift in the equilibrium between free ammonia and ammonium ion in favor of the free ammonia as the pH increased. The free ammonia was felt to be the actual toxic substance.

McCarty and McKinney (18)(19) also studied the effect of other cations on anaerobic waste treatment. They found that for acetate utilizing methane bacteria, the following levels of cations could be tolerated on a daily feed basis at a 30-day solids retention time and at an organic loading of 2 grams per liter per day of acetic acid: Sodium = Potassium = 0.2M, Calcium = Magnesium = 0.175M, ammonium = 0.15M. In addition, it was shown that the toxicity of a slug addition of up to 3500 mg/l of sodium could be antagonized by the presence of 500 to 1000 mg/l of either magnesium or calcium.

The results of studies on cation effects in anaerobic waste treatment detailed above indicate that the methane bacteria exhibit the same basic responses to the presence of cations as all other organisms. They appear to exhibit a greater sensitivity to the toxic effects of cations than the acid formers. This result is not unexpected in view of the greater sensitivity of methane bacteria versus acid forming bacteria to most other environmental conditions. However, the relative toxicity of divalent versus monovalent to methane organisms is rather unusual. On a molar basis the cations studied by McCarty and McKinney (18) showed little difference in toxicity. For most other organisms divalent cations were found to be significantly more toxic than monovalent cations. For example, Winslow and Haywood (6) found that calcium and magnesium were ten times as toxic as sodium and potassium for E. coli on a molar basis.

It should be noted that all previous studies on cation effects were carried out in essentially uncontrolled complex media such as sewage sludge or various trade wastes. Complicating effects from the presence of various antagonists, complexing agents and precipitants may well have caused this shift in the relative toxicity of the cations studied. In order to negate as much as possible these effects, the study reported here was conducted using a specially designed chemically defined medium containing as low a concentration as possible of all extraneous materials. It was felt that use of this medium would yield results which could be accepted as essentially free of complicating effects.

B. Experimental Procedures

1. Introduction

The purpose of this study was the determination of ion effects in the anaerobic waste treatment process. Previous investigations indicated that the methane fermentation stage was much more sensitive to ion effects than the acid production stage. Consequently, the investigations in this study were all carried out solely on the methane fermentation stage.

In order to separate these two phases, all bacterial cultures used were fed only acetic acid and its salts, over a long enough time to eliminate for all practical purposes the presence of acid forming organisms. Acetate was chosen as the only volatile acid fed, because the study by Jeris and McCarty (20) showed that a minimum of 70% of the methane produced in the anaerobic digestion of proteins, fats, and carbohydrates is produced by fermentation of acetic acid.

A wide variety of ions can be present in wastes. These include light metals, heavy metals, and various anions. Previous work has indicated that with respect to ion effects, cations play the predominant role; thus this study was restricted to a determination of cation effects. In order to eliminate slight variations due to anion effects, all salts were added in the form of chlorides. Chloride salts were used because they are very soluble, are neutral, thus eliminating pH effects, and the chloride anion is the least toxic of all anions.

The cations chosen for study were sodium, potassium, calcium, magnesium, and ammonium. These cations are the most prevalent cations occurring in natural waters, are always present in domestic sewage sludge, and are present in high concentrations in many industrial wastes. In addition previous studies on ion effects in anaerobic waste treatment had indicated that the relative effect of these cations on methane bacteria was somewhat at variance with their effect on most other bacteria.

The experimental procedures used were established to fulfill the following two conditions as closely as possible:

1. The digester medium was to contain as low a concentration as possible of all ions, non-ionic growth factors, and any other materials which could cause complicating effects, while still maintaining satisfactory growth of the organisms under study.
2. The digesters were to be operated using procedures similar to those under which anaerobic waste treatment systems are operated in field installations.

The method utilized in this study involved addition of salts to actively fermenting cultures of acetate utilizing methane bacteria. Two general methods were used -- slug feed and daily feed. In the slug feed studies, mixed liquor containing methane bacteria was transferred to Warburg flasks containing appropriate quantities of salts. The rate of reaction was determined from gas production data. The slug feed studies approximated conditions where a shock load of salts gains entrance to a digester.

The second method involved a gradual increase in salt concentrations in units containing methane organisms over a period of several days. This was accomplished by adding the salts to the substrate being fed daily to the bacteria. These studies approximated conditions where a waste being treated always contains a high concentration of ions. These daily feed studies indicated the possibility of adaption to ion concentrations which are inhibitive on a slug feed basis.

The procedures followed for both the slug feed studies and daily feed studies are described below.

2. Procedure for Slug Feed Studies

Five laboratory scale digesters of eight liter ($0.28FT^3$) capacity were started using digested sludge obtained from the Nut Island Sewage Treatment Plant, Boston, Massachusetts, as seed material. All units were operated on a fifteen-day biological solids retention time, at an organic loading of 0.5 grams per liter (0.031 lb. per FT^3) per day of acetic acid; and were maintained at a temperature of 35^oC.

Acetic acid was used as the sole organic substrate to eliminate the presence of all organisms other than acetate utilizing methane bacteria. The 533 ml daily feed was pumped to the digester continuously over a 24 hour period. Mixed liquor was wasted from the digester once per day. Each digester was completely mixed by means of gas recirculation. Figure 1 illustrates a typical digester and its appurtenances.

These units were operated for several months during which time the original seed material was, for all practical purposes, flushed out. During this period, the original material was replaced by a medium whose characteristics are given in Table 1. All studies in this investigation were carried out using this chemically defined medium.

Five separate buffer materials were used in this investigation. These were the bicarbonates of sodium, potassium, ammonium, calcium and magnesium. In any particular digester only one of these bicarbonates served as the buffering material. The buffer level was chosen as 2500 mg/l as calcium carbonate. This level was sufficient to hold the pH at 7.0 without producing an excessive concentration of any cation. At this buffer level, the concentration of the associated monovalent cations would be 0.05 M and of the divalent cations would be 0.025 M.

TABLE 1

GROWTH MEDIUM UTILIZED FOR STUDY OF CATION EFFECTS ON ACETATE UTILIZING METHANE BACTERIA

Chemical	Concentration
Cambridge Tap Water	1000 ml
Bicarbonate Buffer	2500 mg/l as C_aCO_3
$FeCl_3$	60 mg/l
$CoCl_2 \cdot 6H_2O$	8 mg/l
Thiamine-HCl	2 mg/l
K_2SO_4	10 mg/l
NH_4Cl	147 mg/l
$(NH_4)_2 HPO_4$	22 mg/l

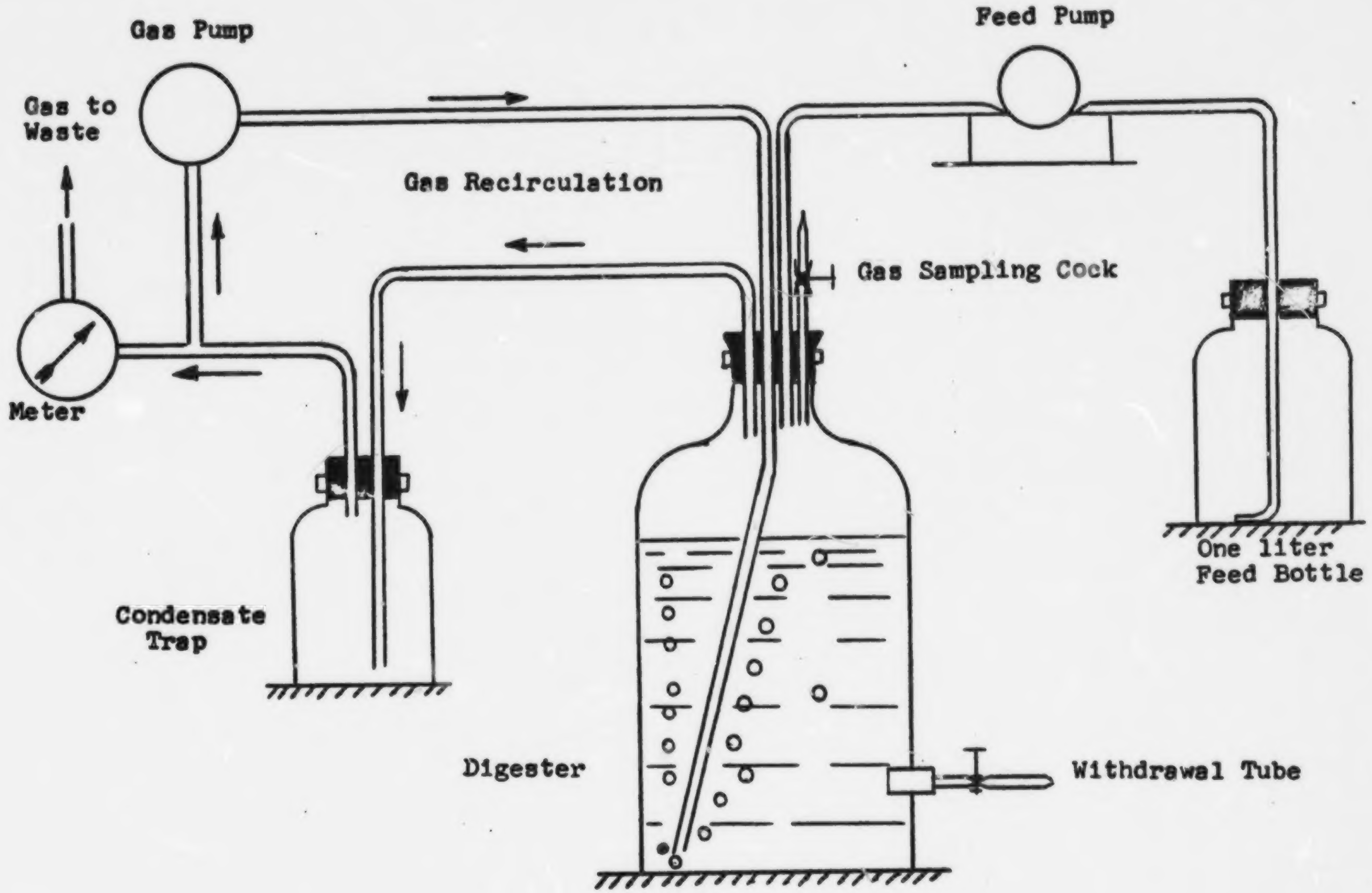

Gas Pump
Feed Pump
Gas to Waste
Gas Recirculation
Gas Sampling Cock
Meter
Condensate Trap
One liter Feed Bottle
Digester
Withdrawal Tube

FIGURE 1 - SCHEMATIC LAYOUT OF CONTINUOUS FEED ACCLIMATION DIGESTER

The quantities of ammonium chloride and ammonium dibasic phosphate used in this medium were chosen based on the requirements of acetate utilizers for nitrogen and phosphorous reported by Speece and McCarty (21), as were the quantities used for ferric chloride, cobalt chloride, and thiamine. At the stated organic loading and solids retention time the quantities used resulted in a 20 mg/l excess of ammonium and a 50 percent excess of phosphorous. The ionic contribution of Cambridge Tap Water to the medium was determined and is given in Table 2.

TABLE 2

IONIC CHARACTERISTICS OF CAMBRIDGE TAP WATER

Ion	Concentration
Sodium	10 mg/1
Potassium	1.8 mg/1
Ammonium	Less than 1 mg/1
Magnesium plus Calcium	50-60 mg/1 as $CaCO_3$

Once the five acclimation digesters were purged of all extraneous material, the cultures they contained were considered ready for studies on ion toxicity. In this study all cation effects were investigated on a slug addition basis. That is, the concentrations of the cations under study were suddenly increased in actively fermenting cultures of methane bacteria. The rate of utilization of the substrate, subsequent to the addition of the cations, was used as the parameter of the cation effect.

The Warburg apparatus was used to measure the rate of substrate utilization. Mixed liquor from the acclimation digesters was transferred to special Warburg flasks containing the chloride salts of the cations under study. The reaction rate was then determined from the rate of methane production. The theory underlying this method has been given previously (18) for constant pressure systems. Although a constant volume study was used in this study, the theory underlying the reaction rate measurements is essentially unchanged. The details of carrying out anaerobic Warburg studies have also been reported previously (22). A summary of this procedure with certain modifications used in this study is given below.

The chloride salts were introduced into the Warburg flasks in solutions made up with distilled water. The flasks were then placed in a drying oven where the salt solutions were evaporated to dryness. After evaporation, the flasks were connected to the Warburg manometers and purged with a mixture of nitrogen and carbon dioxide for a period of approximately two hours. During the purging period, a volatile acids analysis was conducted on the acclimation digester from which mixed liquor was to be transferred. Based on this analysis, the volatile acid concentration of the acclimation unit was raised to approximately 500 mg/l by the addition of acetic acid. A gas analysis was then run on the acclimation unit and the ratio of nitrogen to carbon dioxide in the purging gases was set equal to the ratio of methane to carbon dioxide in the gas in the acclimation digester. After the purging was completed, 50 ml of mixed liquor from the acclimation unit was transferred anaerobically to each flask. These procedures were adopted to eliminate any effect that would result from dilution of the bacterial cultures if the salts and feed were maintained in solution in the Warburg flasks.

3. Procedure for Daily Feed Studies

The daily feed studies were carried out in one liter laboratory scale digesters filled to the 0.75 liter mark. A diagram of a typical digester and its associated gas collection and measurement apparatus is shown in Figure 2. Each digester consisted of a one-liter narrow mouth pyrex bottle. A rubber stopper was inserted in the top of the bottle. Through the rubber stopper, three glass tubes were inserted. One glass tube which was bent and which had one end covered with a rubber tube and pinch clamp, was extended about two-thirds of the way below the liquid surface. This tube served for withdrawal of samples of the digester contents. The other two tubes extended only a short distance below the bottom of the stopper. One which was equipped with a rubber tube and clamp served for the introduction of feed and to expel gas. The third tube was connected by a long rubber tube to a gas collection tube.

The gas collection tubes were made of glass of approximately uniform inside diameter except for narrowing at the ends. These tubes were filled with a saturated water solution of sodium chloride acidified with sulfuric acid. The acid proportion was 15 percent by volume of concentrated sulfuric acid. This acid salt solution has a low solubility for gases. Methyl orange was added to the solution to facilitate observation of the level of the acid-salt solution.

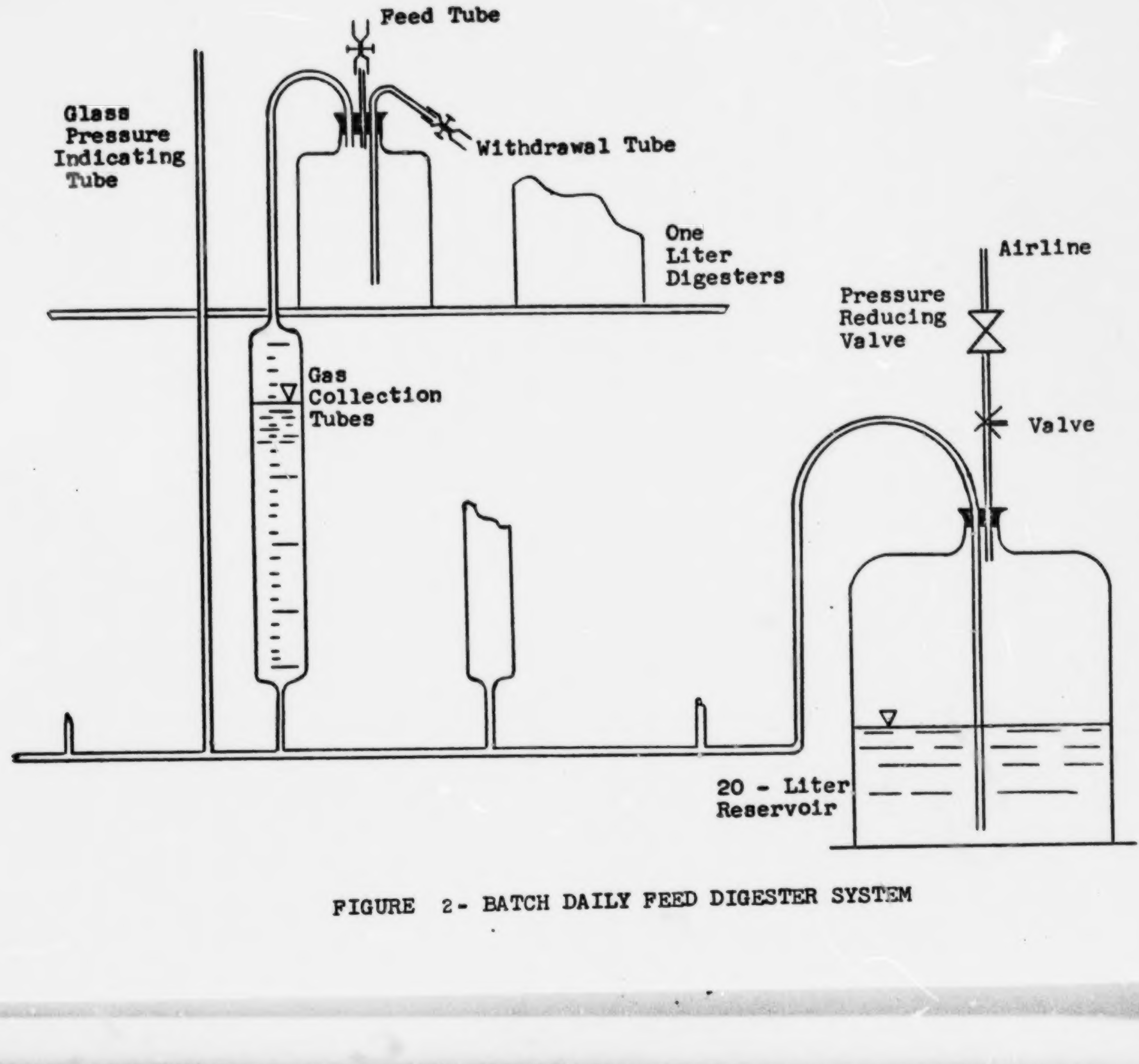

Feed Tube
Glass Pressure Indicating Tube
Withdrawal Tube
One Liter Digesters
Airline
Pressure Reducing Valve
Gas Collection Tubes
Valve
20 - Liter Reservoir

FIGURE 2- BATCH DAILY FEED DIGESTER SYSTEM

Volume calibrations were marked on a piece of wide white adhesive tape pasted to the side of the gas tube.

The confining fluid of a series of gas collection tubes was contained in a 20-liter reservoir. This reservoir was connected to the gas collection tubes by a manifold header system. By the use of a three way valve, air from a pressure tank could be introduced to the reservoir until the pressure produced in the gas collection digester system equalled atmospheric pressure. This was achieved when the confining fluid level in the gas collection tube equalled that in a vertical glass pressure indicating tube. Thus, gas readings at atmospheric pressure could be obtained. By the use of this system the pressure could be increased so that samples could be withdrawn, and vacuum applied so that liquid could be added to the digester.

The daily feed studies were carried out in an incubation room at 35°C and were operated on a 15-day detention time. The digesters were not mixed, but were operated on a once daily feed and waste schedule. The procedure for sampling and feeding:

1. The unit was thoroughly shaken to mix the contents.
2. The pressure system was adjusted and the gas production read.
3. The pressure was raised and the unit thoroughly shaken.
4. A 50 ml sample was withdrawn into a 50 ml graduated cylinder.
5. The gas was expelled to the minus 50 graduation on the gas collection tube.
6. The pressure was lowered to place the system under vacuum.
7. The feed solution made up in a 50 ml graduated cylinder was drawn up into an open tipped 50 ml pipet. A separate pipet was used for each set of units.
8. The pipet was inserted in the digester feed tap and the clamp opened to allow the feed solution to flow into the unit. Care was taken to close the clamp as soon as the last drop of solution left the pipet to prevent the entrance of air into the unit.
9. The unit was thoroughly shaken to mix the feed with the other contents of the unit.

The units used were started from a 1:2 mixture of screened, digested sludge and Cambridge Tap Water. On the first day of operation, the alkalinity in the units was adjusted to 2500 mg/l by the addition of the proper amount of acetate salt. The cation used depended on the cation which was to be used as the buffer cation in the unit.

After the initial adjustment of the alkalinity, the addition of the normal feed to the digester was commenced. The normal feed used was a growth medium identical to that used in the acclimation digesters of the slug feed studies.

The units were operated under these conditions for three months before the runs were begun. This provided a sufficient number of detention times to remove all the material originally present. During this three month period, the operation of the units was not always satisfactory. After the first two weeks, the rate of utilization began to decline. This condition continued for the next two weeks. Then, some mixed liquor was transferred from the acclimation digester to these daily feed units. Over the next few weeks the operation gradually improved. During the last month before the runs were started, the operation of all units was satisfactory.

Once the digesters had been purged of all the original seed salts and sludge, the digesters were considered ready for experimental studies. Five runs were then conducted to determine if the results from the slug feed studies were applicable to normal conditions of operation, involving daily feed over an extended period of time. The feed solutions fed in each run will be described under experimental results.

C. Results of Slug Feed Studies

Thirty separate Warburg Runs were conducted to determine the effect of the cations; sodium, potassium, ammonium, calcium, and magnesium, both singly and in combination on methane fermentation. These runs were conducted with the following purposes in mind:

1. The determination of the effect of individual cation concentration (Runs 1 to 5).
2. The determination of antagonistic or non-antagonistic relationships in dual cation systems (Runs 6 to 26).
3. The determination of antagonistic relationships in multi-cation systems (Runs 27 to 30).

Run 1 - Sodium Inhibition

In this study various quantities of sodium chloride were added in Warburg studies on mixed liquor from the sodium acclimation digester. The mixed liquor from the sodium acclimation digester was utilized to eliminate effects from the presence of ions other than those present in the basic growth medium. Duplicates were utilized for all sodium concentrations tested. Two units contained no added sodium chloride, and served as controls. The concentration of sodium in these controls was 0.05M as sodium served as the buffer cation.

The results of this study are shown in Figure 3, where the average of the duplicates is plotted. These results indicate that the sodium ion did not show significant inhibition until the concentration increased above 0.2M. Beyond 0.2M, the inhibition increased almost linearly with concentration. The average reaction rate of the controls was 474 mg/l/day.

Run 2 - Ammonium Inhibition

This study was carried out in exactly the same manner as Run No. 1, except that the ammonium chloride was added to mixed liquor from the acclimation digester with an ammonium buffer.

The results of this study are shown in Figure 4. The results indicate that inhibition starts between 0.075 and 0.1M ammonium with a definite inhibition apparent at concentrations greater than 0.1M. At first the inhibition increases linearly with ammonium concentration, but then starts to level out. A sharp increase in inhibition is noted between 0.3 and 0.4M ammonium. At 0.4M the unit was for all practical purposes dead. A considerable amount of acclimation occurred in the units

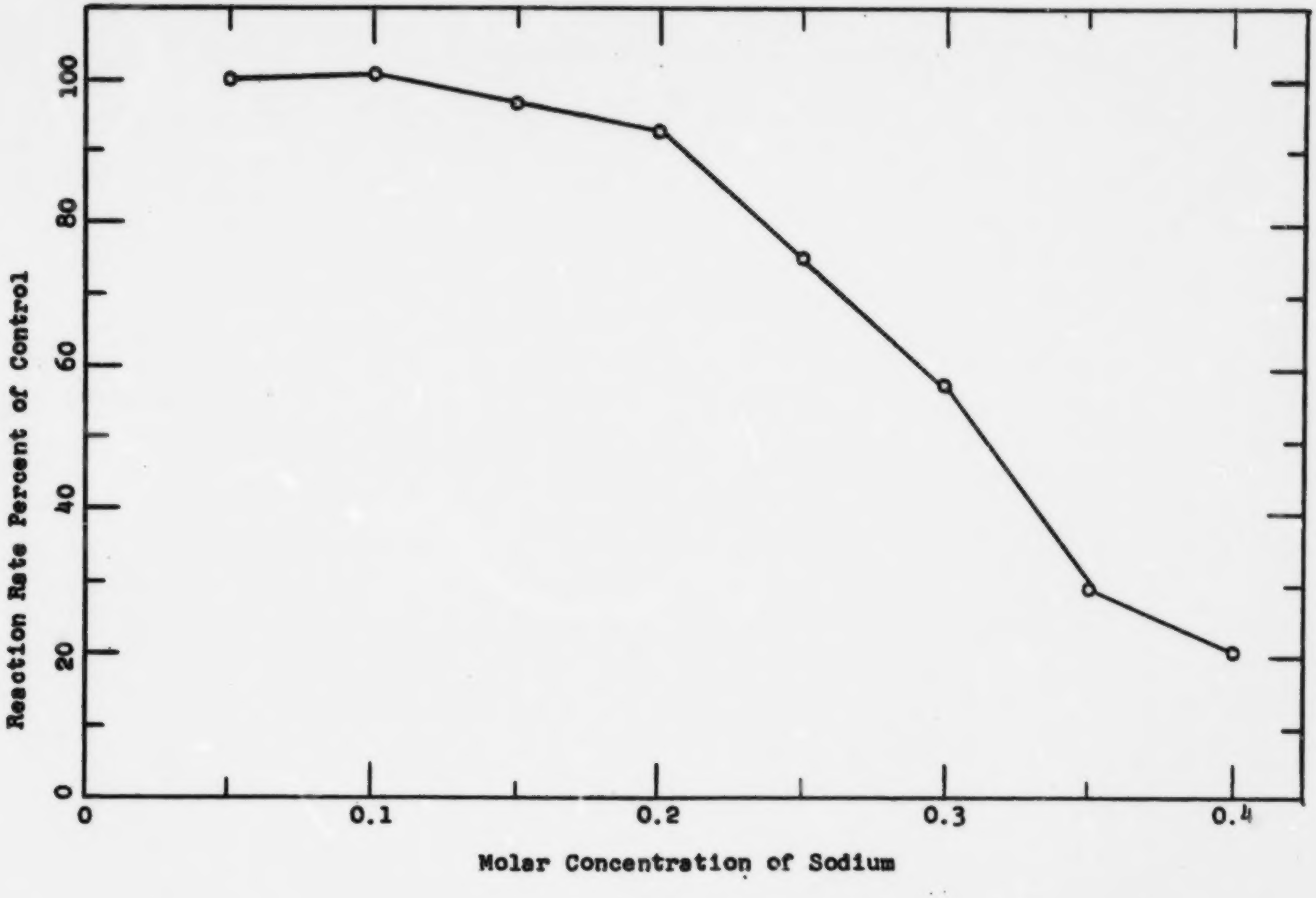


FIGURE 3 - EFFECT OF SODIUM CONCENTRATION ON RATE OF ACETATE UTILIZATION

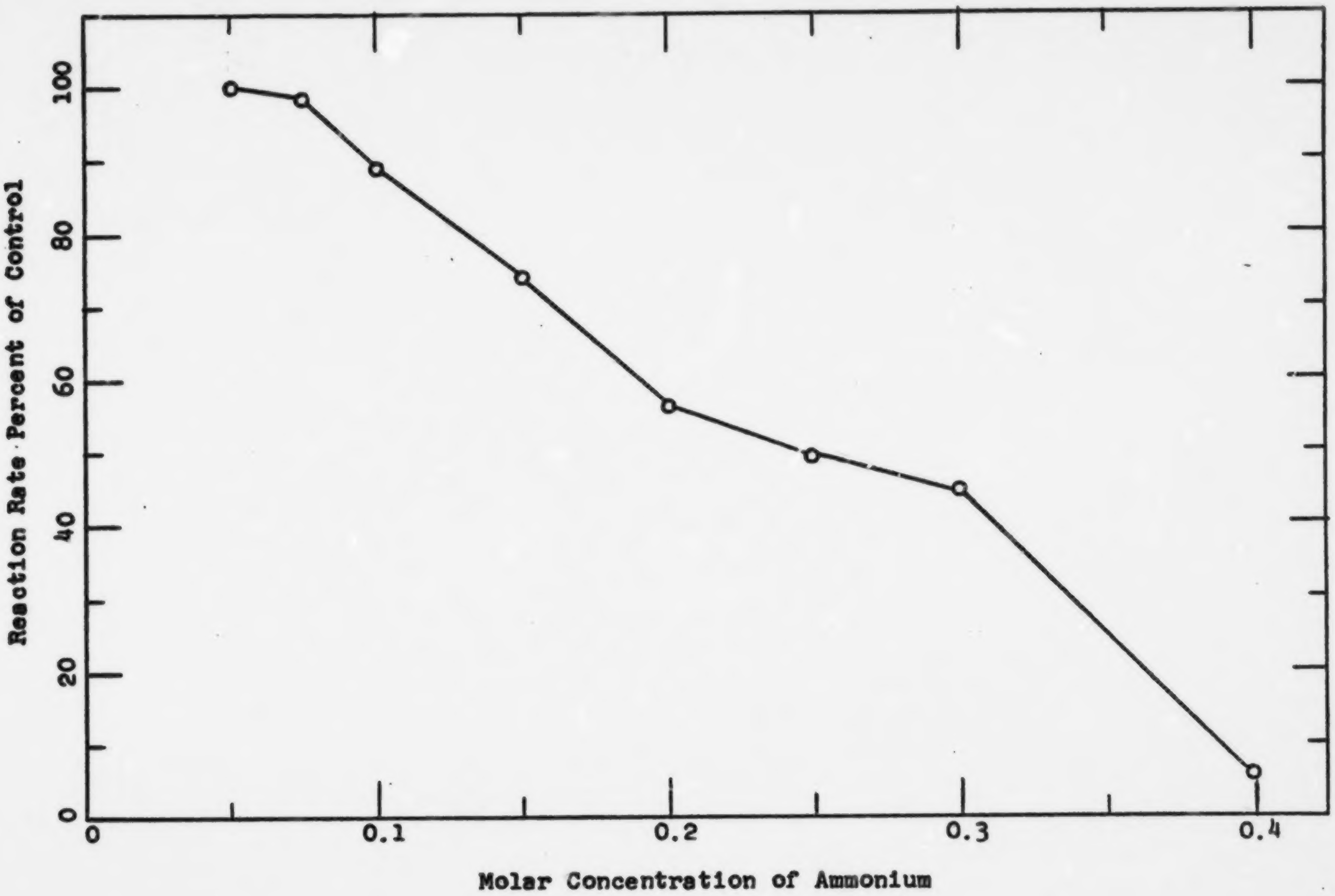


FIGURE 4- EFFECT OF AMMONIUM ON RATE OF ACETATE UTILIZATION

containing 0.3M ammonium, which may account for the flattening of the inhibition curve near this value. Again, the agreement between duplicates was good. The results plotted in Figure 4 are the averages of the duplicates.

Run 3 - Potassium Inhibition

This run was carried out in the same manner as Runs 1 and 2. It was noted in most runs that plots of gas production vs time were straight lines. It was found that this was not so when high concentrations of potassium were present. In this situation the rate of gas production showed a definite fall-off during the run. This is illustrated in Figure 5. It can be seen that at concentrations greater than 0.1M the curves of gas production versus time are made of two straight line sections. Even for a concentration showing a definite acclimation curve, after about half of the substrate was utilized, a change in the rate occurred. In determining the rate of reaction, the rate from the latter section of the curve was used because this section more closely approximates what eventually will occur in a unit subjected to a shock load of potassium.

The data showing reaction rate as a function of potassium concentration is presented in Figure 6. These results indicate that potassium shows approximately the same inhibition as ammonium at low concentrations, but is more inhibitive at high concentrations. The pattern of inhibition also is similar to that of ammonium; an approximately linear increase of toxicity at first followed by a leveling off at high concentrations.

Run 4 - Magnesium Inhibition

The same procedures were employed in this run as the first three runs. The control contained only 0.025M magnesium, as this is the quantity necessary to provide a buffer of 2500 mg/l.

The results of this run are shown in Figure 7. These results show that magnesium is more inhibitive than the monovalent ions investigated in Runs 1 to 3. Inhibition is evident at the first increment of magnesium above that in the control. Significant inhibition is evident at concentrations greater than 0.05M. The pattern of inhibition shows at first an approximately linear increase with magnesium concentration which levels off when extreme inhibition is achieved. At magnesium concentrations greater than 0.2M, complete inhibition was evident.

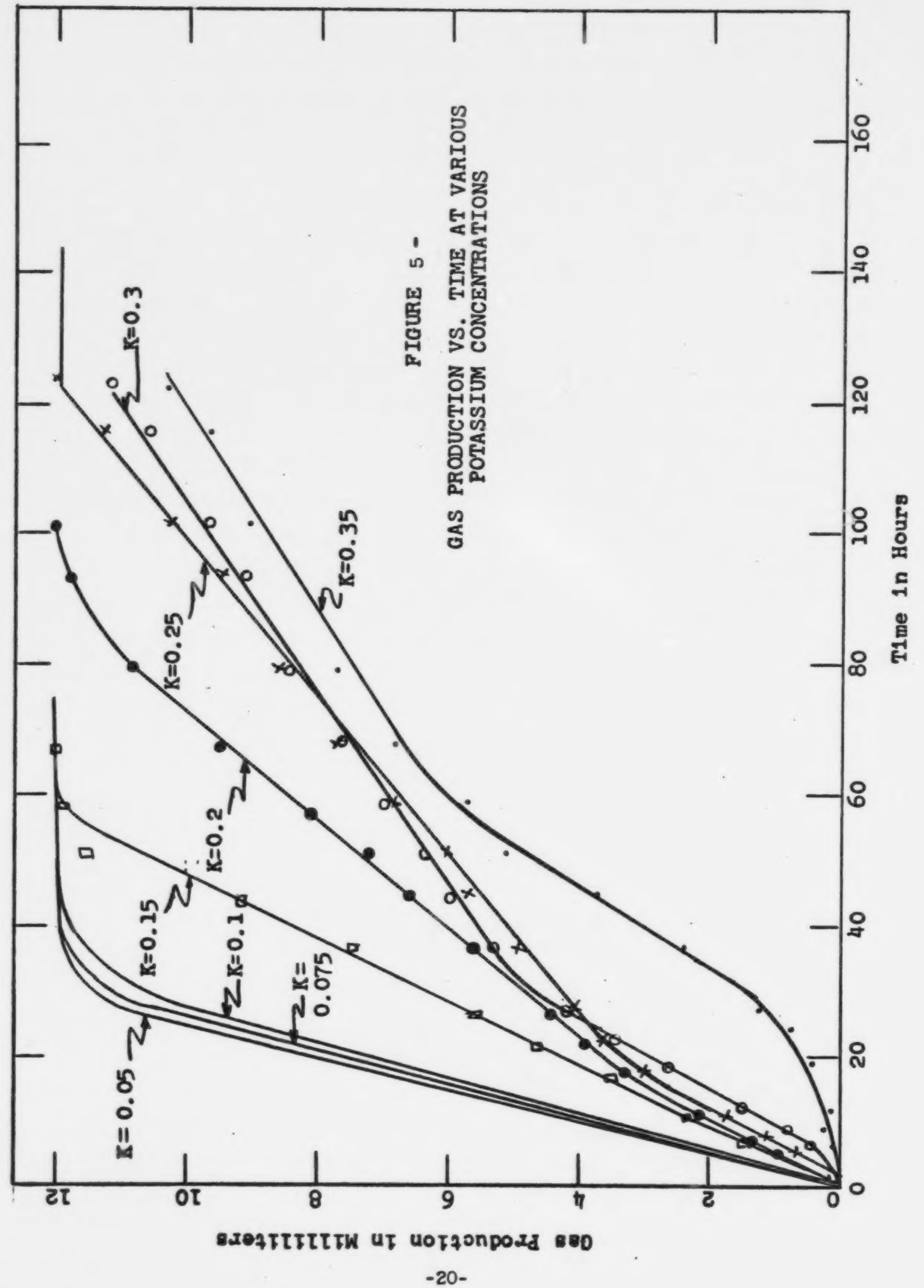


FIGURE 5 -

GAS PRODUCTION VS. TIME AT VARIOUS POTASSIUM CONCENTRATIONS

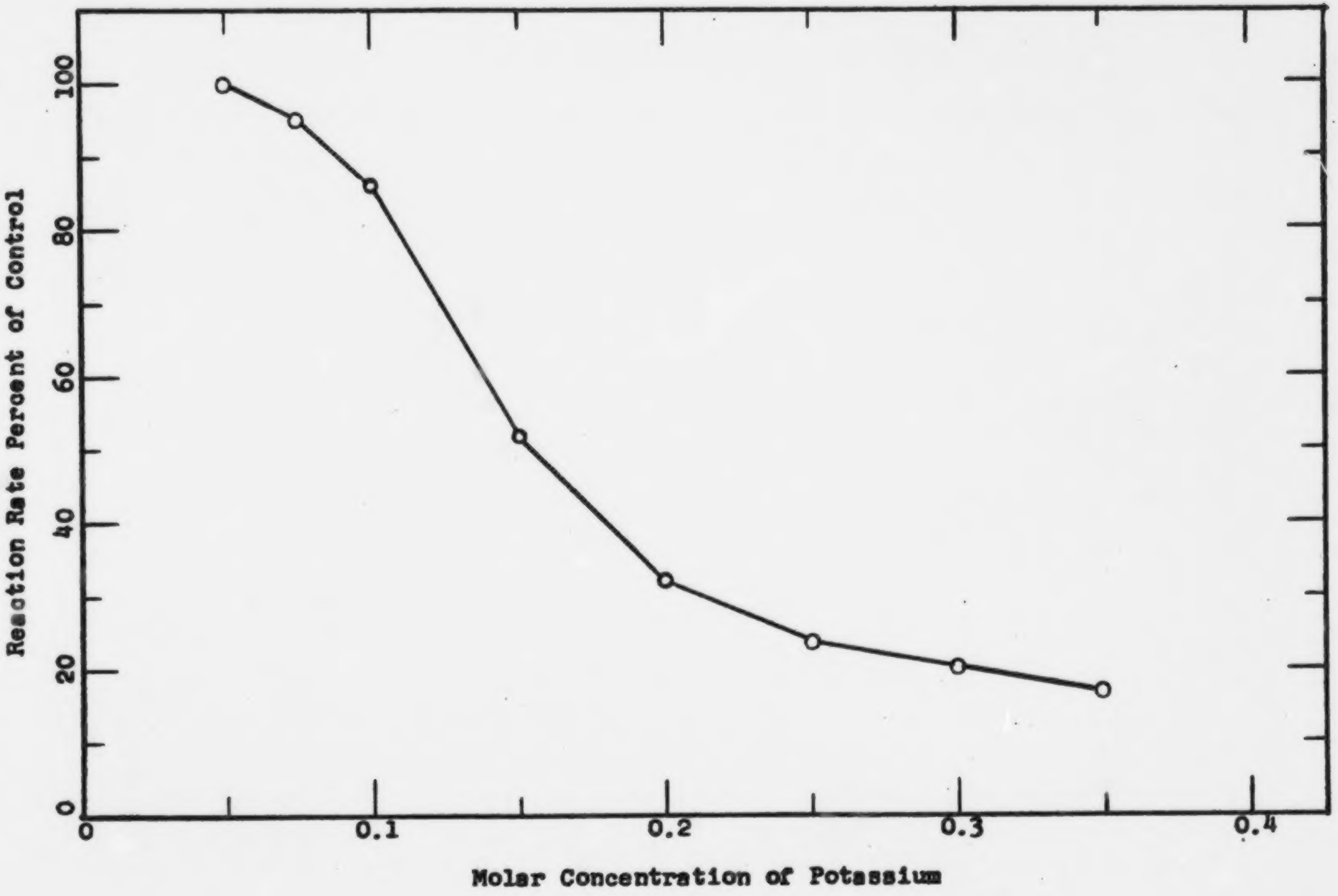


FIGURE 6 - EFFECT OF POTASSIUM CONCENTRATION ON RATE OF ACETATE UTILIZATION

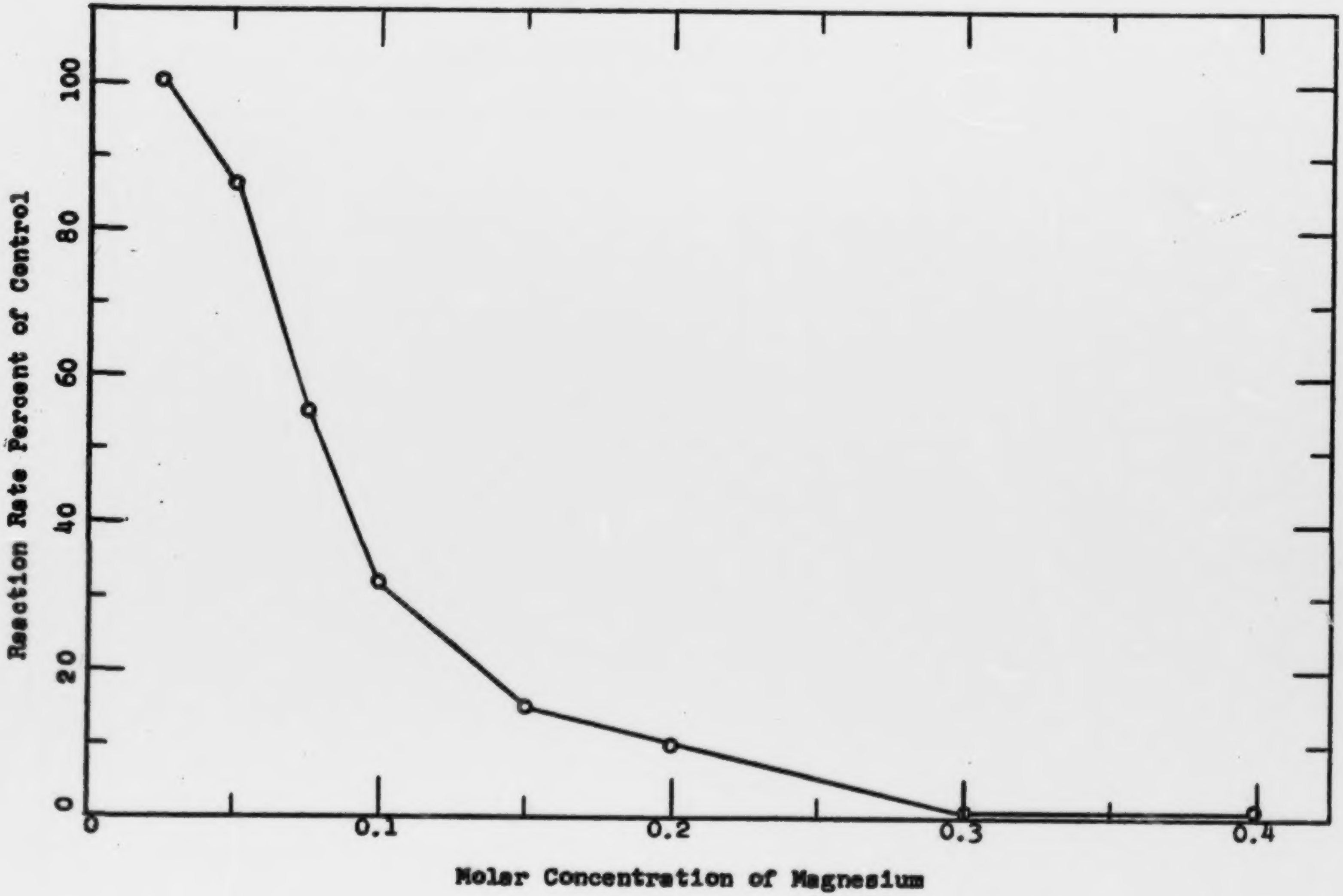


FIGURE 7 - EFFECT OF MAGNESIUM ON RATE OF ACETATE DEGRADATION

It is possible that pH effects may account for some of the inhibition produced by high magnesium concentrations. The pH of the mixed liquor in the flasks where the magnesium concentrations were 0.3M and 0.4M were 6.55 and 6.45, respectively. These low pH values were undoubtedly the result of the precipitation of magnesium bicarbonate at high magnesium concentrations. At magnesium concentrations below 0.3M, however, the pH values of the mixed liquor were in the proper range. Thus at magnesium concentrations below 0.3M, the inhibition produced was wholly due to the magnesium cation.

Run 5 - Calcium Inhibition

The procedures employed were the same as those in the first four runs. The controls had a theoretical calcium concentration of 0.025M. However, because of the low solubility of calcium bicarbonate, the actual concentration was less. An estimate of the actual calcium in solution can be obtained from the alkalinity value at the beginning of the run, which was 1900 mg/1 instead of 2500 mg/1.

The results are shown in Figure 8. These results show that calcium is much more inhibitive than the monovalent ions studied, but not quite as inhibitive as magnesium. Toxicity is evident at all calcium concentrations above the control with appreciable inhibition evident at 0.075M. The inhibition appears to increase linearly with concentration at values of calcium greater than 0.75M, but the curve flattens out at concentrations where extreme inhibition is evident. At concentrations greater than 0.2M, complete inhibition is evidenced. As in the case of magnesium, pH effects play a role in calcium toxicity at high calcium concentrations. The pH fell below 6.5 at concentrations of calcium greater than 0.2M. However, the results show that the major inhibitive effect of calcium was achieved at concentrations below 0.2M. Thus, as with magnesium, the major inhibitive effect must be ascribed to the calcium cation.

Run 6 - Effect of Potassium on Sodium Inhibition

The purpose of this run was to determine the ability of potassium to antagonize the inhibitory effect of sodium. The concentration of sodium was raised to inhibitory levels of 0.3M and 0.4M in the mixed liquor from the sodium acclimation digester, and various quantities of potassium ranging from 0 to 0.12M were introduced.

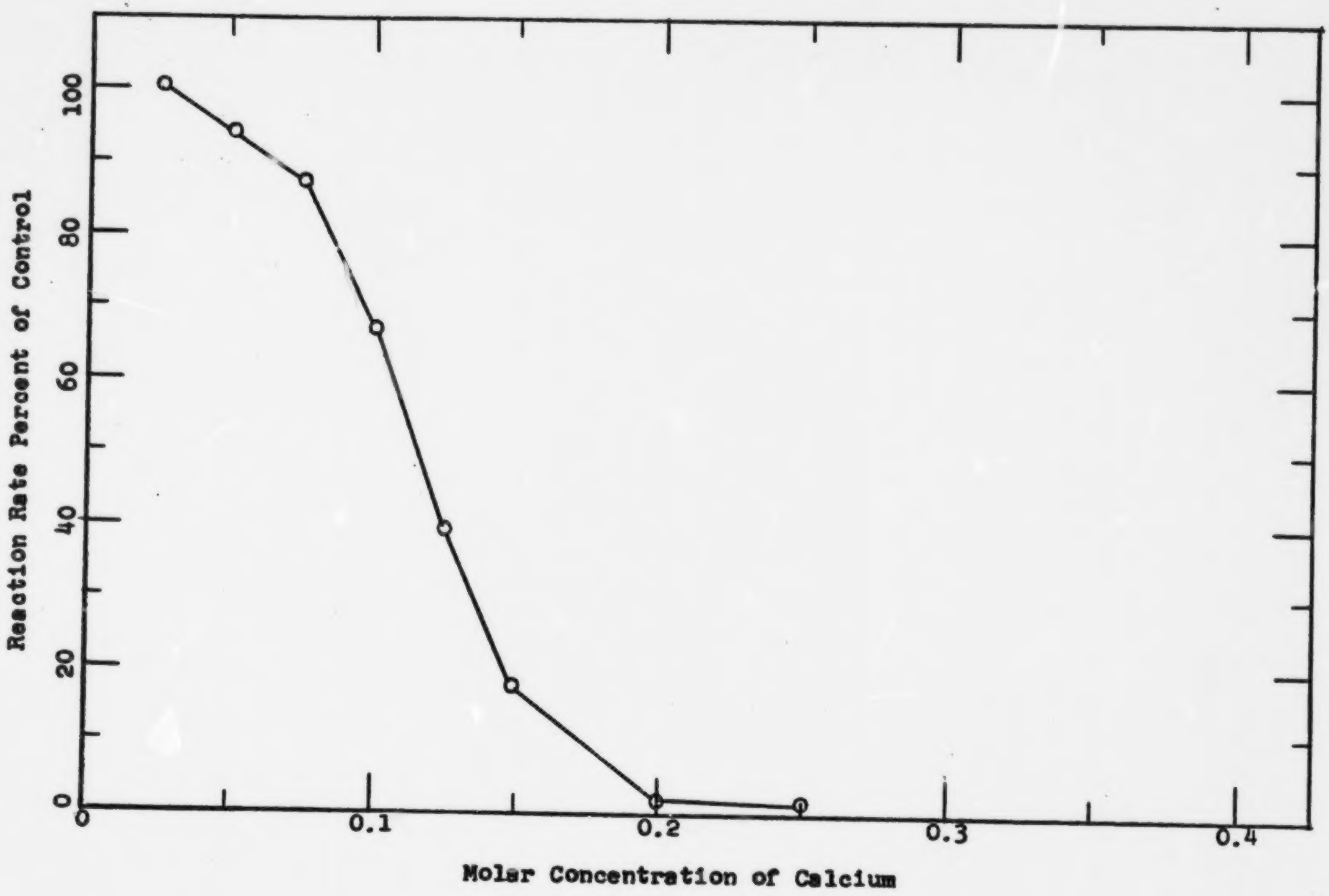


FIGURE 8 - EFFECT OF CALCIUM CONCENTRATION ON RATE OF ACETATE UTILIZATION

The results are shown in Figure 9. The results show a marked ability of potassium to alleviate the inhibition of sodium. At a concentration of 0.3M sodium only 2 x 10^{-3}M potassium raised the reaction ratio from 54.0 to 73 percent of the control. The antagonism achieved remained essentially constant up to 0.06M potassium, and then started to decrease. Between 0.1 and 0.11M potassium the reaction rate equalled that of the unit containing no potassium. At the higher level of sodium maximum antagonism was not achieved until the potassium concentration reached 5 x 10^{-3}M. In this case the peak was sharper ranging from 5 x 10^{-3}M to 3 x 10^{-2}M. As the potassium increased past the peak, the antagonism decreased linearly. The crossover point was reached between 0.11 and 0.12M potassium.

Run 7 - Effect of Ammonium on Sodium Inhibition

The purpose and procedures used in this study were the same as in Run 6, except for the use of ammonium ion as the antagonist, and the range of ammonium concentrations used. The range of ammonium used was 0 to 0.1M.

The results are shown in Figure 10. These results indicate not only that ammonium cannot antagonize sodium, but that as the ammonium concentration increases, the inhibition of the combination increases. This occurs even at values of ammonium which are not inhibitive when ammonium is the only ion in the system. The increased toxicity appears to be almost additive as the reaction rate is 22 percent of the control in the presence of 0.3M sodium plus 0.1M ammonium, while it is 18 percent of the control in the presence of 0.4M sodium alone. Another point to note is that the shape of these curves is similar to those for the antagonism of sodium by potassium, if consideration is taken of the portion beyond the point of peak antagonism in the sodium-potassium system.

Run 8 - Effect of Calcium on Sodium Inhibition

The purpose of this run and the procedures used were the same as those in Runs 7 and 8. The calcium concentrations used ranged from 0 to 0.1M.

The results of this study are shown in Figure 11. These results indicate a very slight tendency of calcium to antagonize sodium at very low calcium concentrations. However, for all practical purposes the antagonism is nil. At the lower concentration of sodium, there is a definite increase in inhibition as the calcium increases past 0.01M. This effect appears to level out at concentrations greater than 0.05M calcium. On the other hand, at the higher sodium

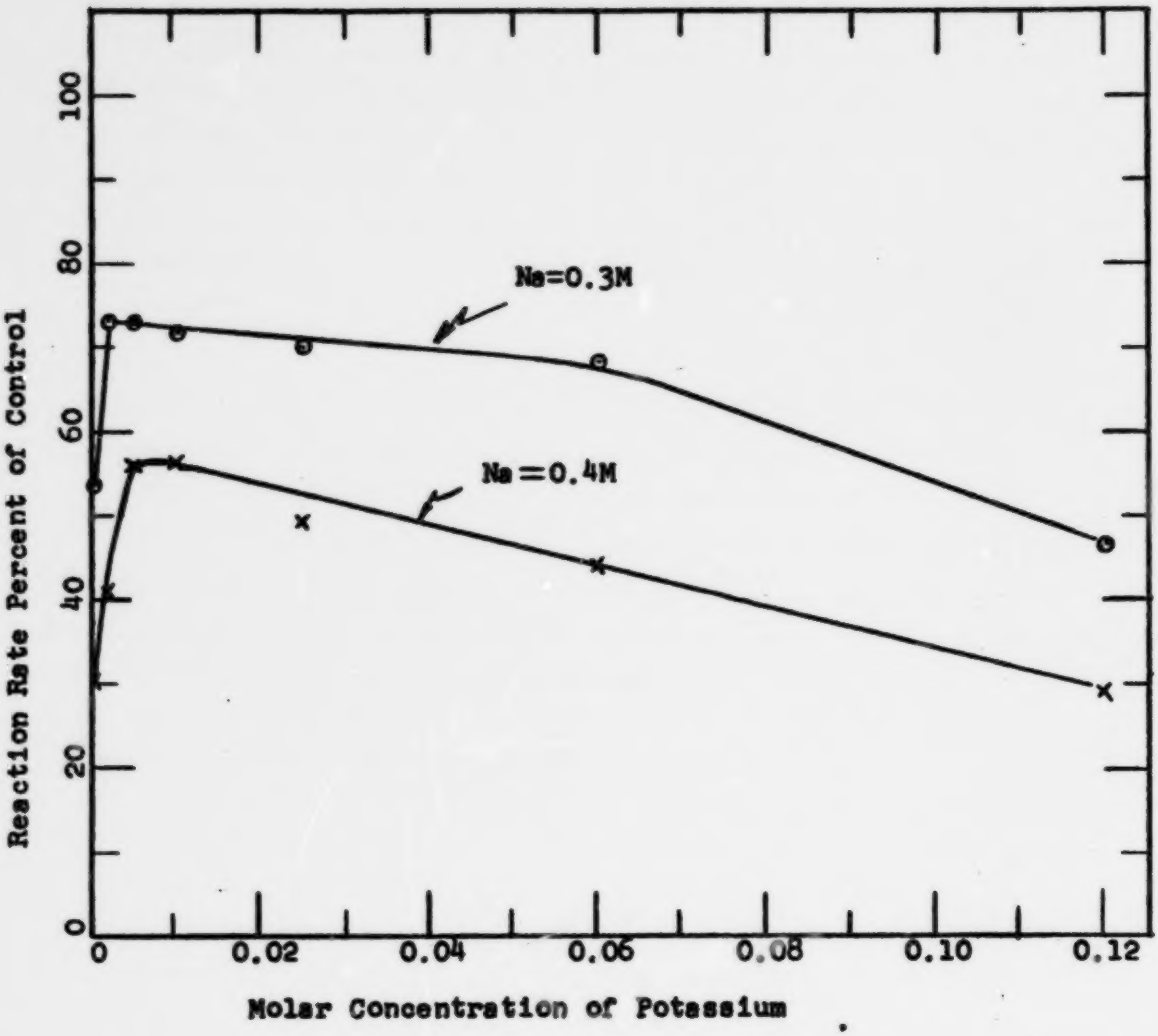


FIGURE 9 - EFFECT OF POTASSIUM ON SODIUM INHIBITION

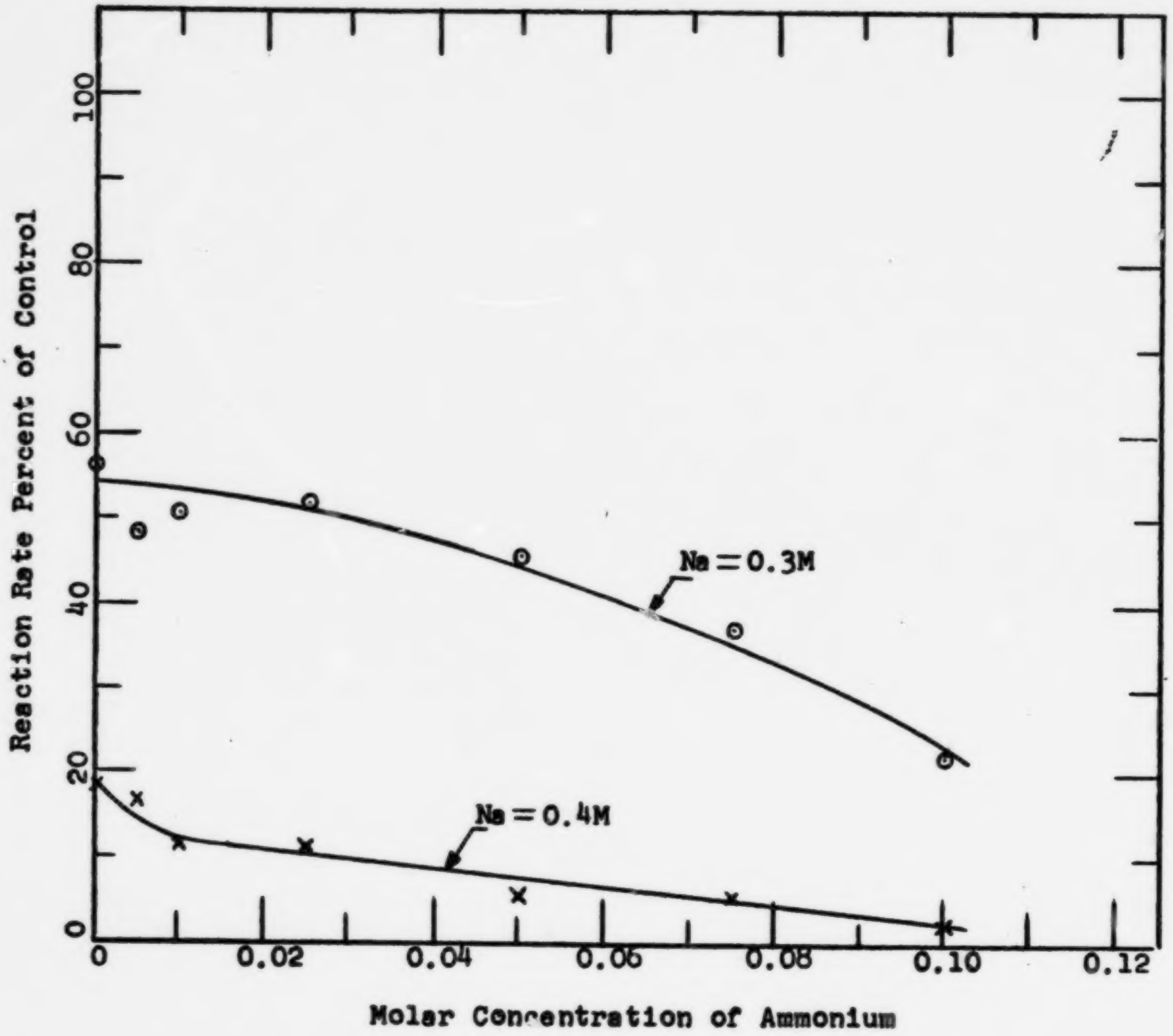


FIGURE 10 - EFFECT OF AMMONIUM ON SODIUM INHIBITION

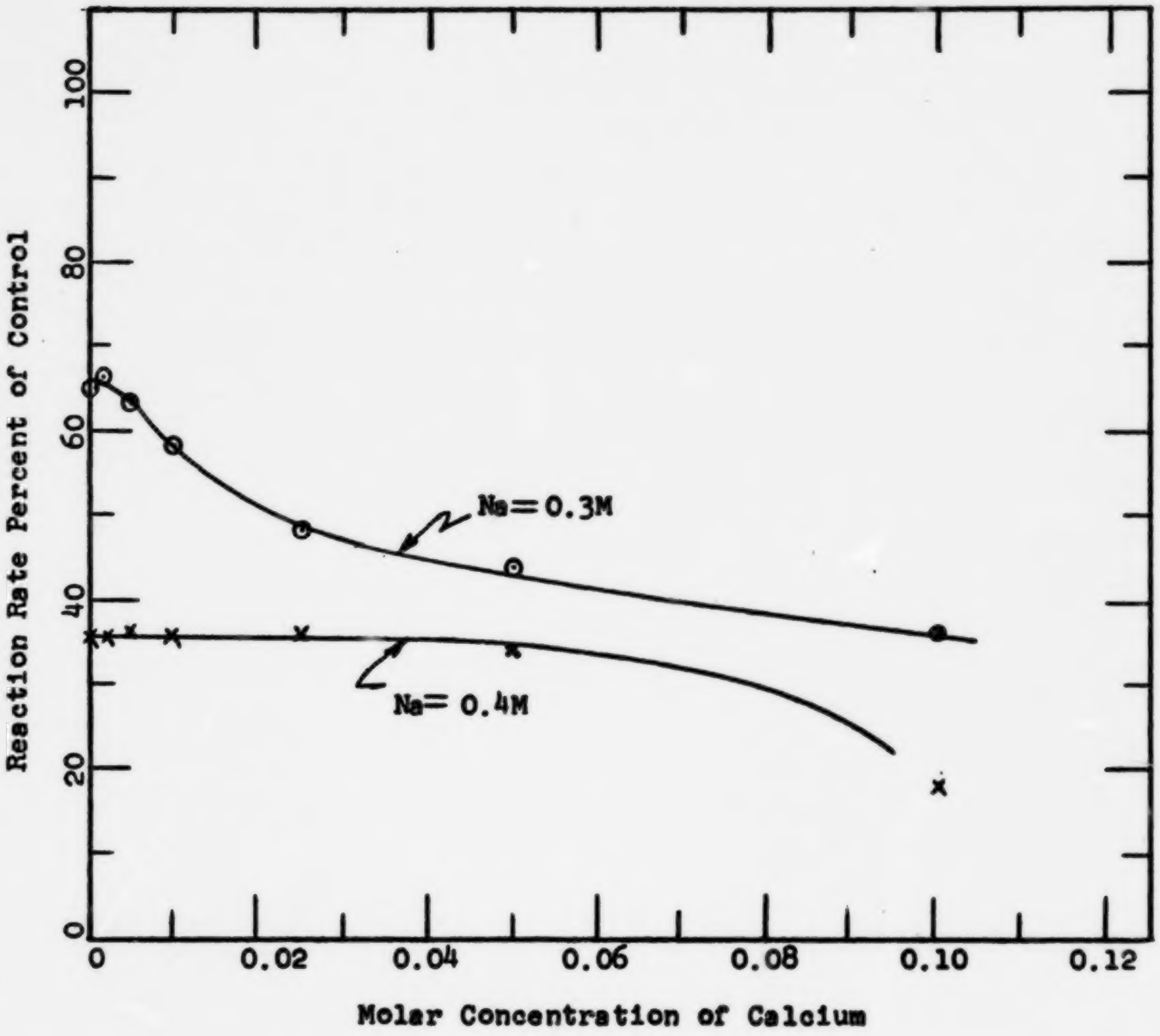


FIGURE 11 - EFFECT OF CALCIUM ON SODIUM INHIBITION

concentration, low concentrations of calcium yield no increased inhibitive effect. An increased inhibitive effect was, however, evidenced once calcium increased beyond 0.05M at the higher sodium concentration.

Run 9 - Effect of Magnesium on Sodium Inhibition

This run was conducted in the same manner as the previous three runs. The magnesium range investigated was from 0 to 0.075M.

The results of this run are shown in Figure 12. It can be seen from these results that magnesium produces an effect similar to calcium. At the lower sodium level a decrease in reaction rate is observed which tends to level off as the magnesium increases. At the higher value of sodium, no increase in inhibitive effect is observed until the magnesium concentration increases beyond 0.05M.

It should be noted that the inhibition of sodium noted in Runs 8 and 9 was less than that observed in Runs 1, 6 and 7. The difference is not great, but may obscure some slight ability of these divalent ions to antagonize sodium inhibition. In general, however, it appears that the divalent ions do not possess much ability to antagonize sodium.

In addition, combinations of these ions with sodium at the concentrations tested, increased the inhibition observed above that for sodium alone. This latter effect appears to reach significant proportions at concentrations of the divalent cations slightly below the concentrations at which the divalent cations would produce inhibition when present alone.

Run 10 - Effect of Sodium on Ammonium Inhibition

The purpose of this run was to determine the ability of sodium to antagonize the inhibition due to ammonium ion. The ammonium concentration was raised to 0.15M and 0.25M in the mixed liquor from the ammonium acclimation digester, and various quantities of sodium were added ranging from 0 to 0.1M.

The results of this run are shown in Figure 13. This data reveals that at low concentrations, sodium is a good antagonist of ammonium. In fact at 0.15M ammonium plus 0.01M sodium, the rate of reaction is greater than the rate of the control. Significant antagonism is achieved between 2 x 10^{-3}M and 5 x 10^{-2}M sodium at an ammonium concentration of 0.15M. As the sodium increased beyond this range, the antagonism decreased. At 0.08M sodium the reaction rate

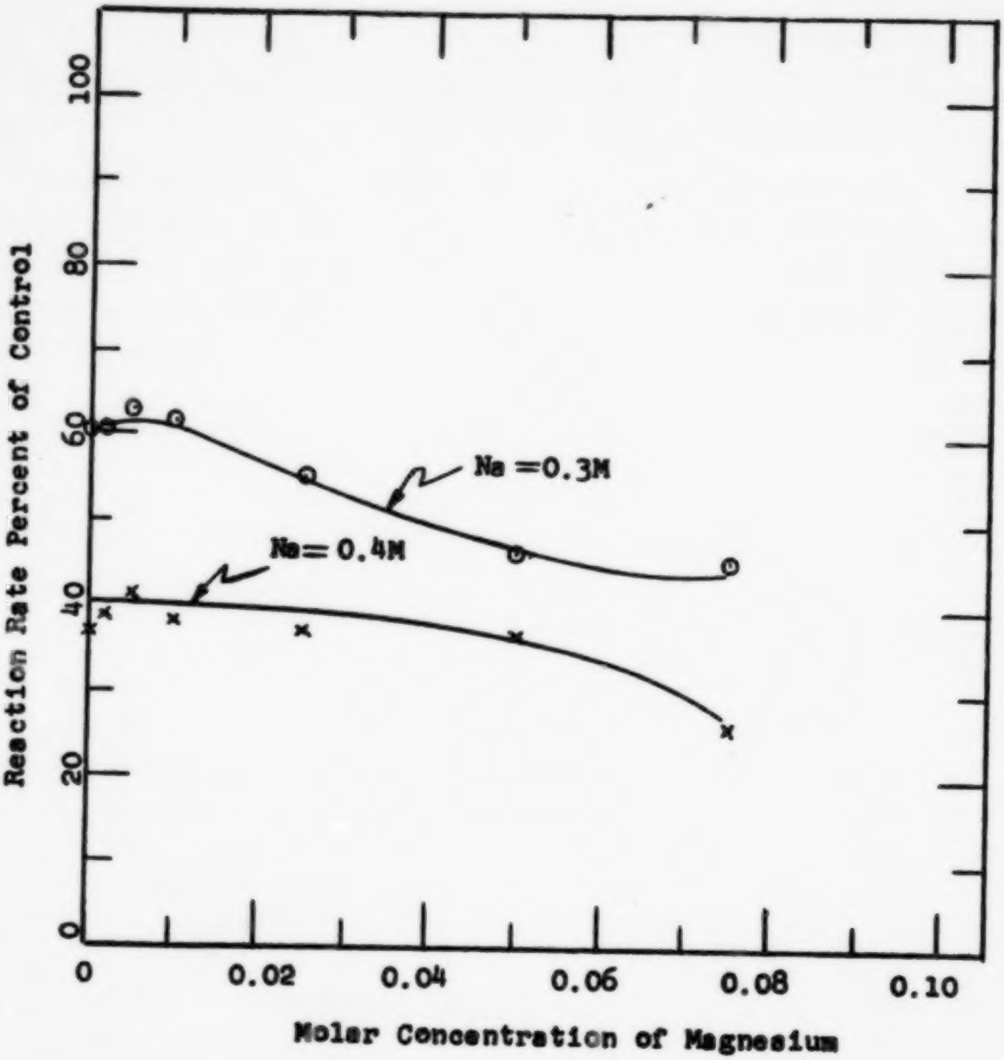


FIGURE 12 - EFFECT OF MAGNESIUM ON SODIUM INHIBITION

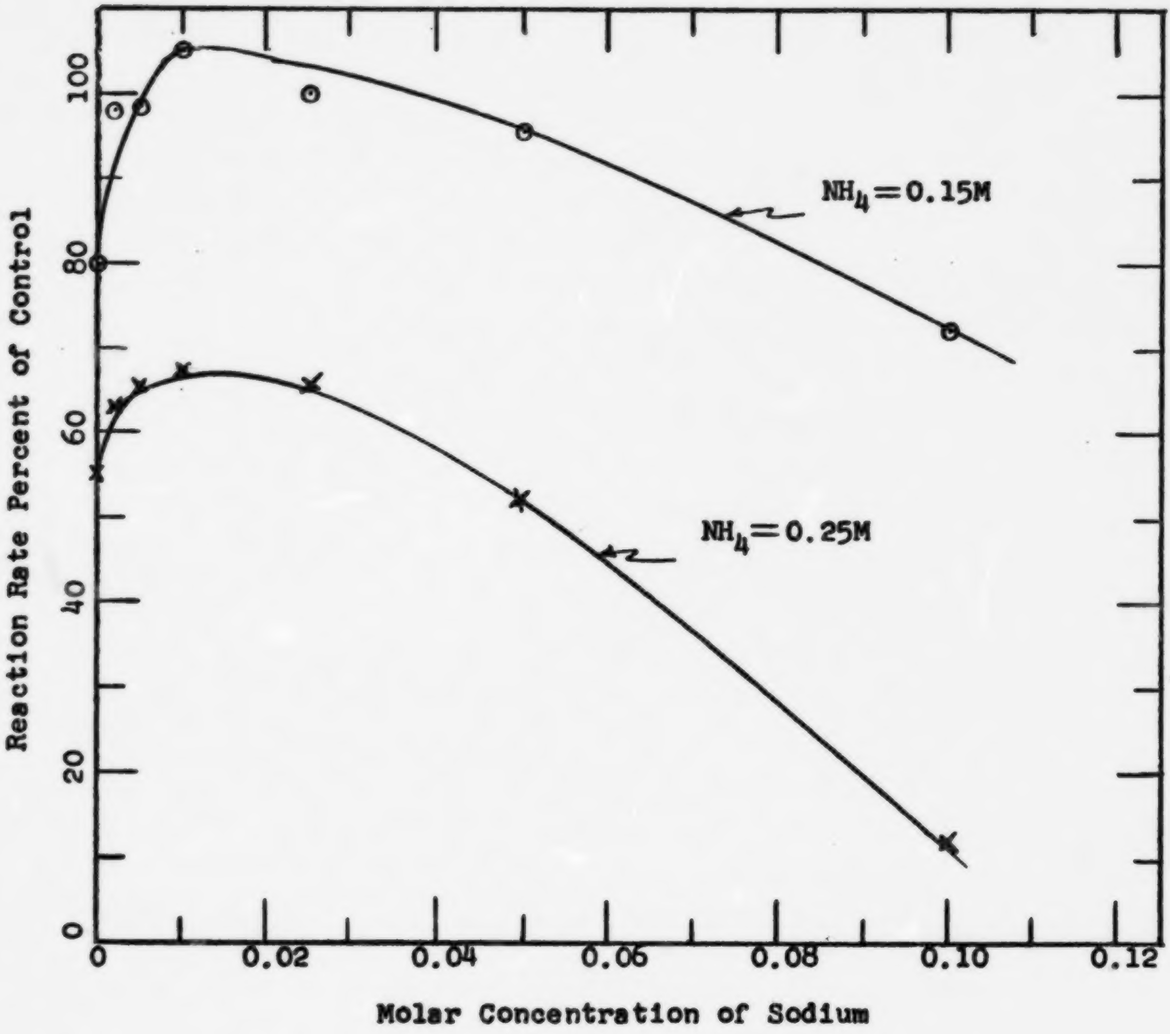


FIGURE 13 - EFFECT OF SODIUM ON AMMONIUM INHIBITION

equalled that of the unit containing no added sodium. At the higher value of ammonium, 0.25M, the range of peak antagonism narrowed to 5×10^{-3}M to 2.5×10^{-2}M. In addition, the cross over point shifted in to 0.045M sodium. Complete inhibition was observed when the sodium reached 0.1M in the presence of 0.25M ammonium. This value is greatly in excess of that observed when either of these ions is present alone.

These data are very significant in that they indicate that the ratio of ions present has very little effect in this system. This is illustrated by the fact that at a sodium to ammonium ratio of 0.4, antagonism is evidenced when ammonium equals 0.15M while complete inhibition is observed when ammonium equals 0.25M. In addition the point of maximum antagonism occurs at 0.01M sodium independent of the ammonium concentration.

Run 11 - Effect of Potassium on Ammonium Inhibition

The same procedure was used in this run as in Run 10. The range of potassium used was 0 to 0.12M.

The results are shown in Figure 14. It is clearly evident that potassium exhibits no ability to antagonize ammonium inhibition. Rather, the presence of potassium increases the apparent inhibition of ammonium. This increased inhibition becomes evident at concentrations greater than 0.025M potassium. This value of potassium is non-inhibitive when it is the only ion present. The curves shown in Figure 14 are practically parallel which indicates that it is the concentration of potassium present which controls the effects observed in this system, rather than the ratio of ions present.

Run 12 - Effect of Calcium on Ammonium Inhibition

The purpose and procedures used in this run were the same as in Runs 10 and 11. The range of calcium concentrations used was 0 to 0.1M.

The results of this run are shown in Figure 15. The results obtained in this run are very similar to those from Run 11. As the calcium increased, the apparent inhibition of the ammonium increased. Significant increases in inhibition were apparent at concentrations of calcium greater than 0.02M. The parallel nature of the curves again indicates that the concentration of calcium present is the dominant factor. The effect seems to be greater than a simple addition of separate effects as the reaction rate in a system containing 0.15M ammonium plus 0.1M calcium is much less than that of a system containing 0.25M ammonium.

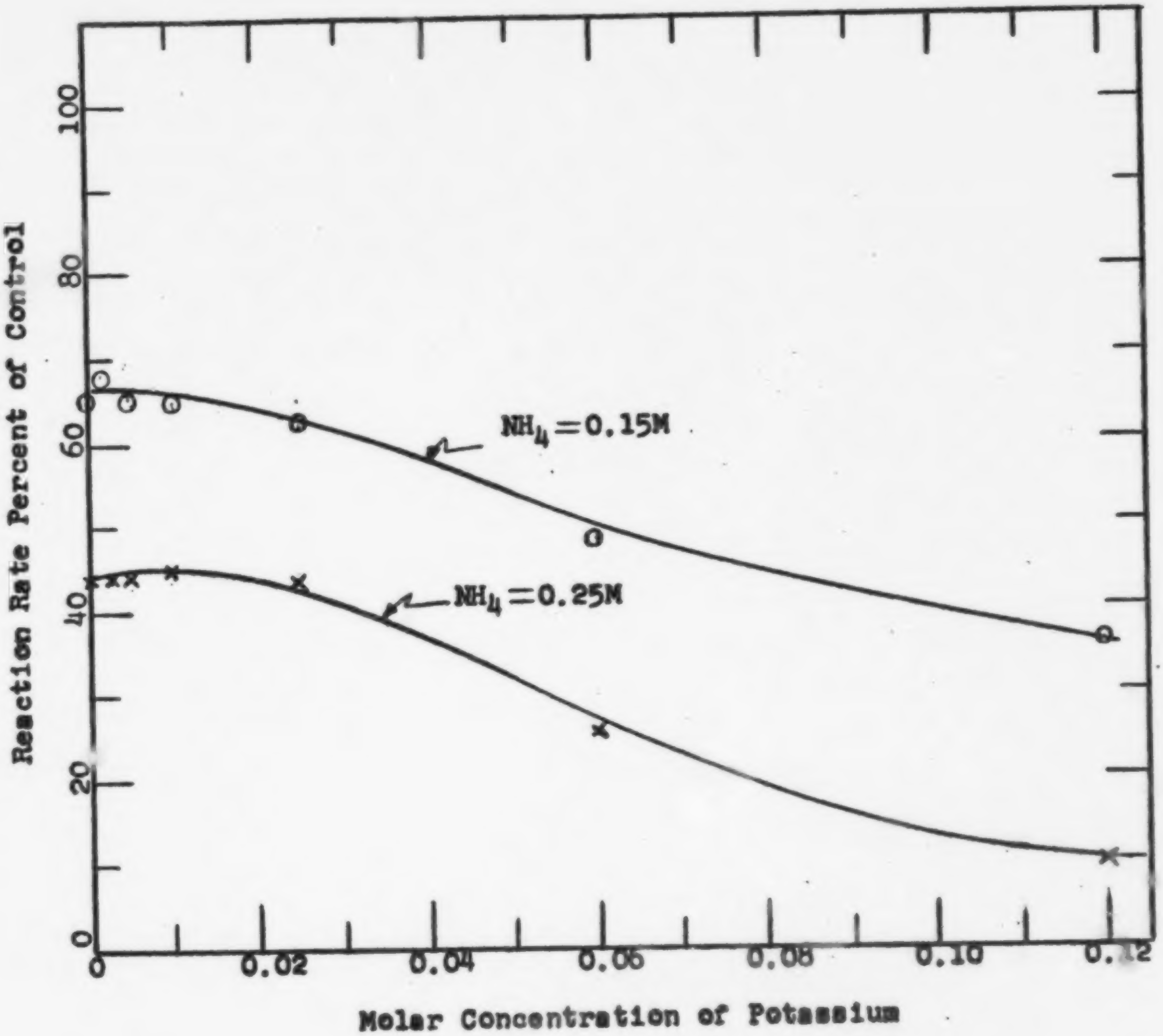


FIGURE 14- EFFECT OF POTASSIUM ON AMMONIUM INHIBITION

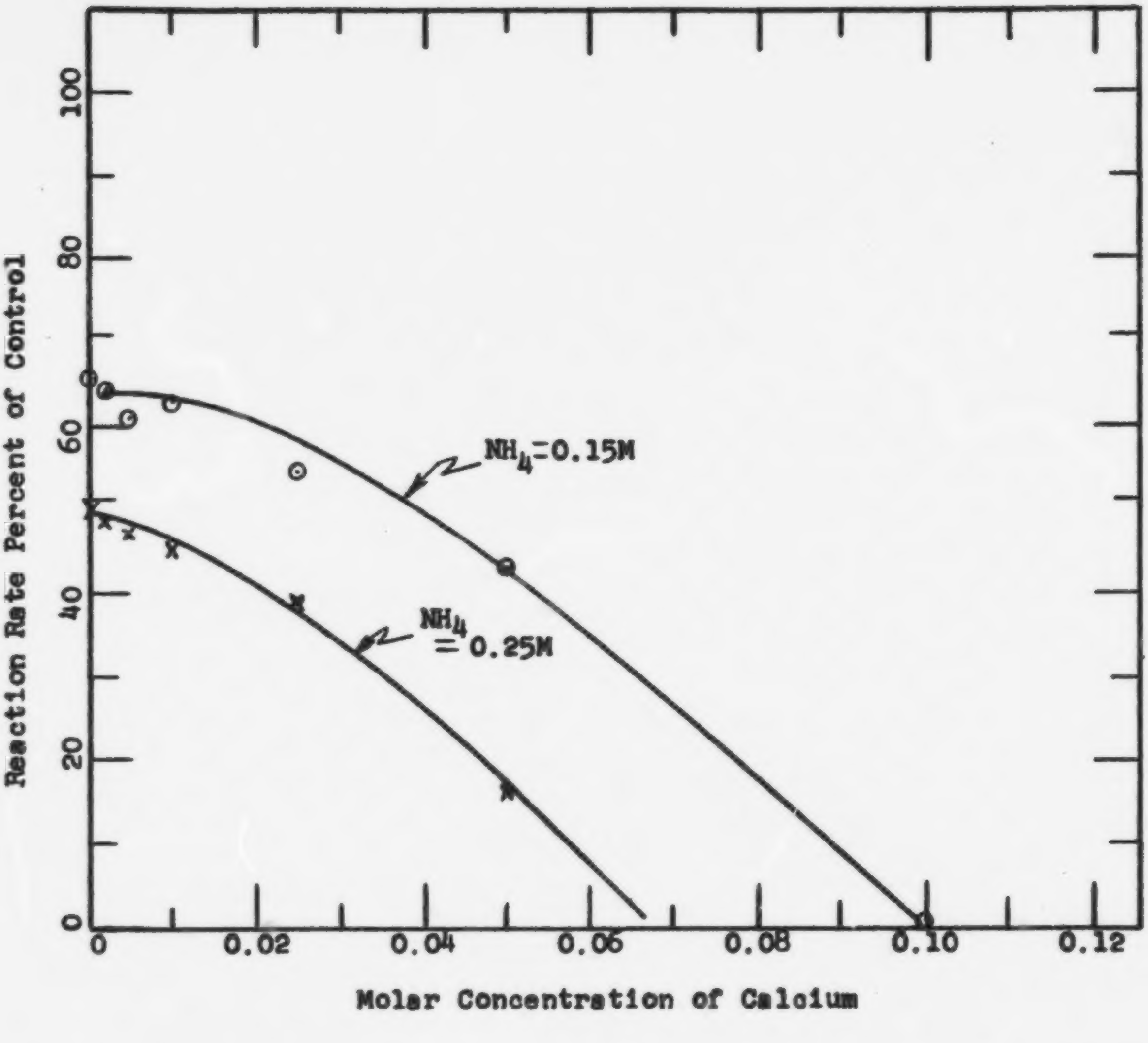


FIGURE 15 - EFFECT OF CALCIUM ON AMMONIUM INHIBITION

Run 13 - Effect of Magnesium on Ammonium Inhibition

Identical procedures and ammonium concentrations were used in this run as were used in Runs 10 to 12. The range of magnesium tested was 0 to 0.075M.

The results of this run are presented in Figure 16. The results obtained are very similar to those obtained in Run 12 for calcium-ammonium mixtures. Inhibition greater than that produced by either cation alone became evident at rather low concentrations of magnesium, approximately 0.01M. The parallel nature of the curves shown in Figure 16 indicates again that the effect achieved by the mixture is a function of the concentration of each cation present, rather than the ratio of the cation concentrations. The results obtained also indicate that the effect observed is not a strictly additive one as 0.15M ammonium plus 0.075M magnesium is more inhibitive than 0.25M ammonium.

Runs 14, 15, 16 - Effect of Sodium and Calcium on Potassium Inhibition

In these three runs the ability of sodium and calcium to individually antagonize potassium inhibition was investigated. The effect of sodium was studied in Run 14 and in part of Run 15. The effect of calcium was investigated in part of Run 15 and in all units of Run 16. The levels of potassium used were 0.15M, 0.25M, and 0.35M. The levels of sodium and calcium used were 0 to 0.1M. In each run the mixed liquor used was from the potassium acclimation unit.

Sodium

The effect of sodium is shown in Figure 17. The data shows that sodium acts as a very good antagonist of potassium up to some concentration of potassium between 0.25M and 0.35M. The pattern of the effect of combinations of sodium and potassium is very interesting. At 0.15M potassium all values of sodium used could accomplish antagonism. Peak antagonism was reached at 5×10^{-3}M sodium and the level of antagonism achieved remained essentially constant up to 0.1M sodium. At 0.25M potassium all values of sodium again showed antagonism. However, the peak was not reached until 0.01M sodium and extended only till 0.05M sodium. At sodium concentrations beyond 0.05M the antagonism decreased. This same type of effect was noted for sodium antagonism of ammonium in Run 10. The effect in Run 10 was more pronouced than in this run. At 0.35M potassium no antagonism was observed; rather all additions of sodium produced a slight increase in inhibition.

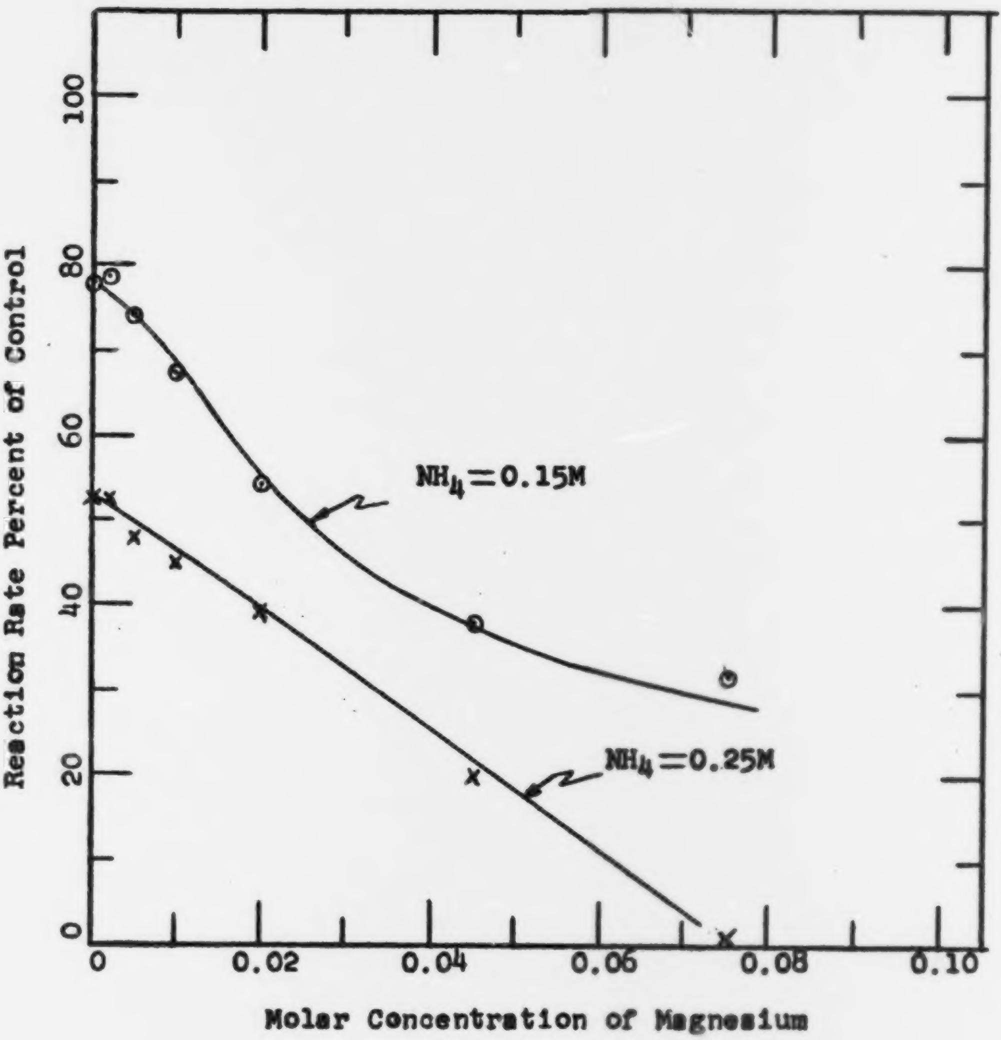


FIGURE 16 - EFFECT OF MAGNESIUM ON AMMONIUM INHIBITION

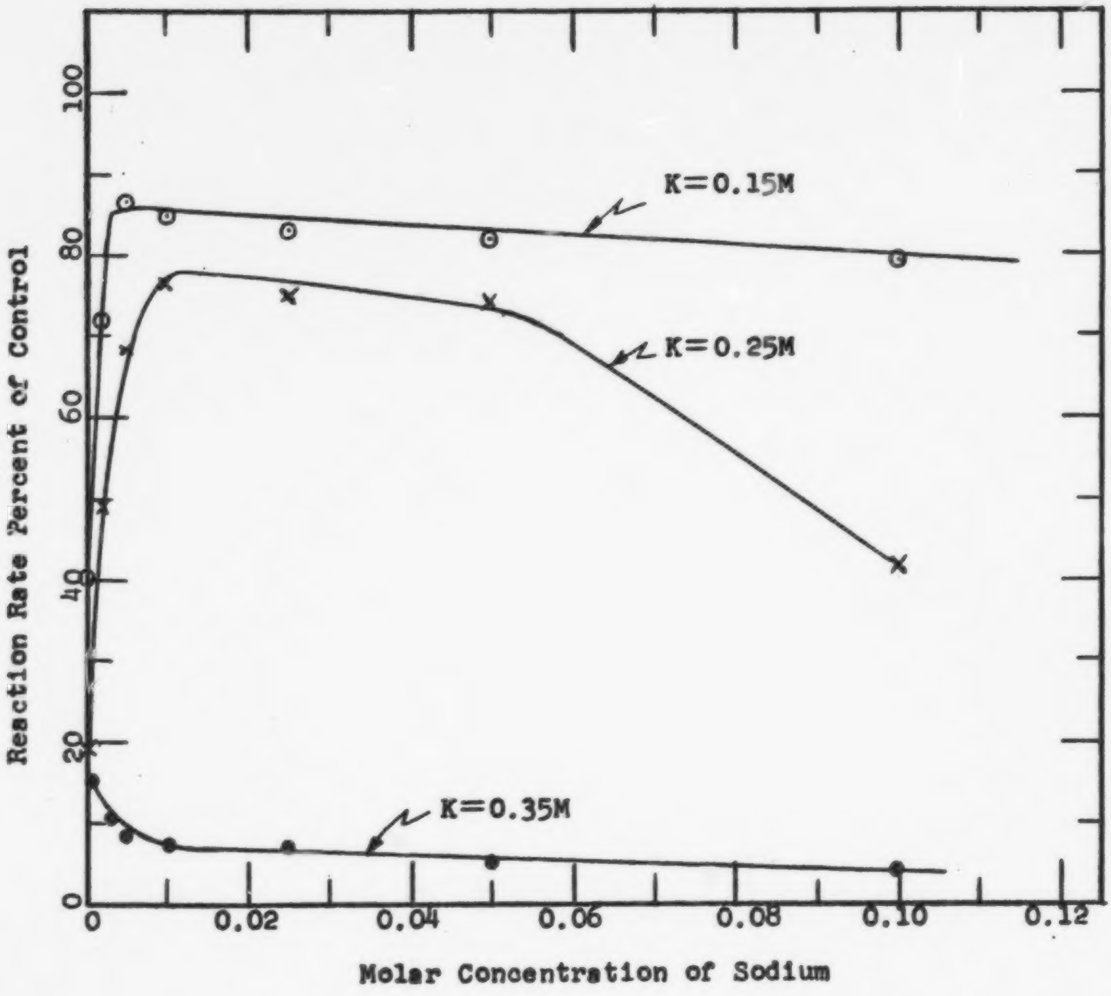


FIGURE 17 - EFFECT OF SODIUM ON POTASSIUM INHIBITION

Calcium

The results of these runs are shown in Figure 18. The results show that at 0.15M and 0.25M potassium, good antagonism was achieved by calcium, but the results were not as good as with sodium. However, at 0.35M potassium calcium was able to antagonize potassium inhibition while sodium was not.

At 0.15M potassium, the antagonism peak was reached at 2×10^{-3}M. The peak was maintained up to 5×10^{-2}M calcium and then showed a slow drop-off. Similar results were obtained at 0.25M potassium with the peak lasting from 5×10^{-3} to 5×10^{-2}M. At 0.35M potassium, a very sharp peak was reached at 5×10^{-3}M calcium and the range of maximum antagonism was restricted to 2×10^{-3}M to 1×10^{-2}M. At calcium concentrations greater than 0.05M the combination was more inhibitive than the presence of 0.35M potassium alone.

Although no cross over point was observed at 0.15M potassium and 0.25M potassium, the trend of the data indicates that the cross over point would occur at the same calcium concentration for both potassium levels. This is similar to the situation for potassium antagonism of sodium in Run 6. However, the shift of the cross over point and narrowing of the antagonism range, observed in previous runs, is also illustrated by this run when consideration is taken of the results at 0.35M potassium.

Runs 17 and 18 - Effect of Magnesium and Ammonium on Potassium Inhibition

In these two runs the ability of ammonium and magnesium individually to antagonize potassium inhibition was tested. In Run 17 the potassium concentration used was 0.15M, while 0.25M potassium was used in Run 18. In both runs the values of magnesium and ammonium used were 0 to 0.075M and 0 to 0.1M respectively.

Magnesium

The effect of magnesium on potassium inhibition is hown in Figure 19. These results show that magnesium can readily antagonize potassium inhibition. The results achieved appear to be intermediate between those of sodium and calcium with respect to the magnitude of antagonism in the peak range. At 0.15M potassium maximum antagonism is achieved at 2×10^{-3}M magnesium, with the peak range extending from 2×10^{-3}M to 0.05M. Antagonism was achieved at all magnesium concentrations tested. As the magnesium increased beyond the peak, the rate of fall-off in antagonism was low.

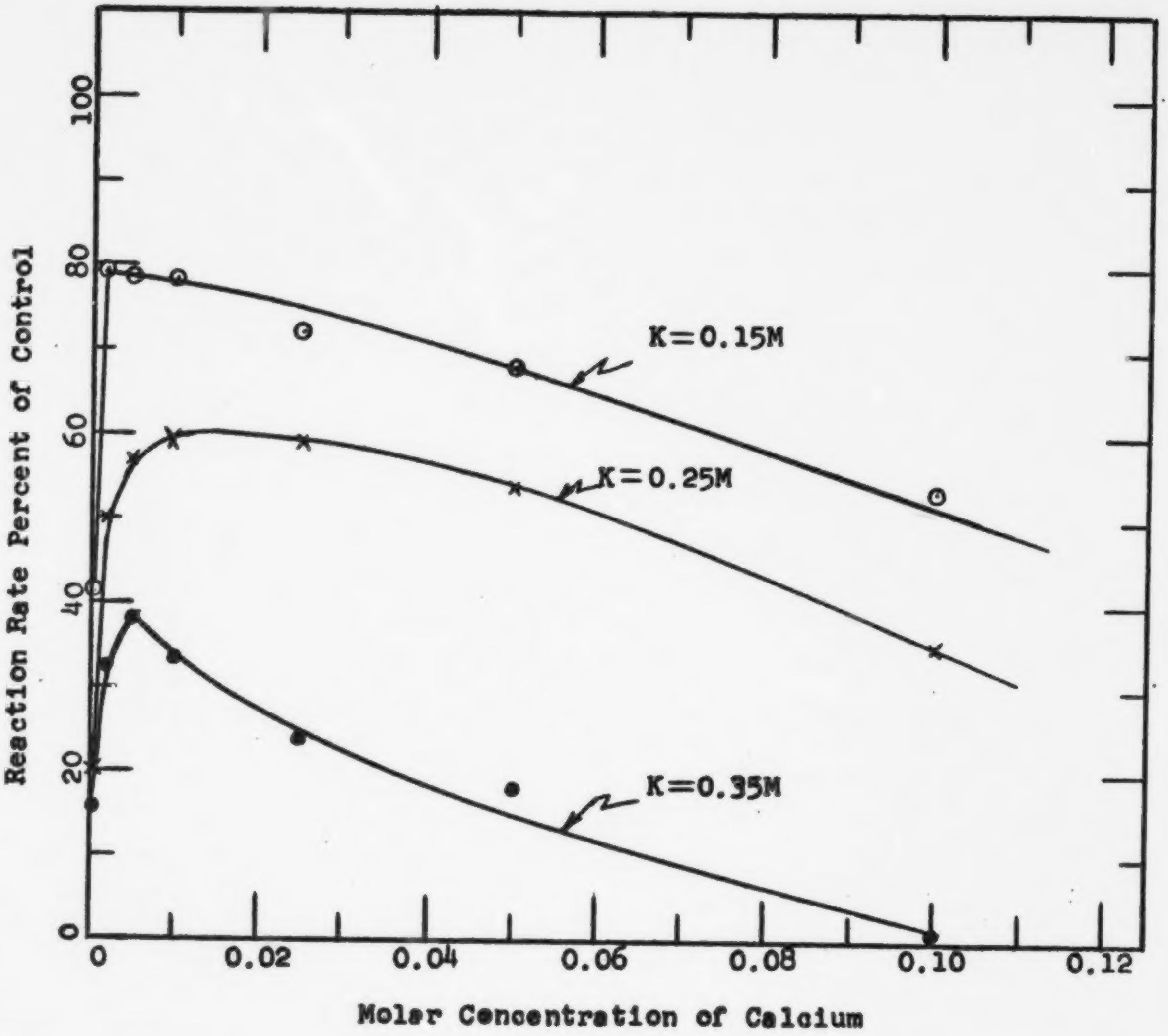


FIGURE 18 - EFFECT OF CALCIUM ON POTASSIUM INHIBITION

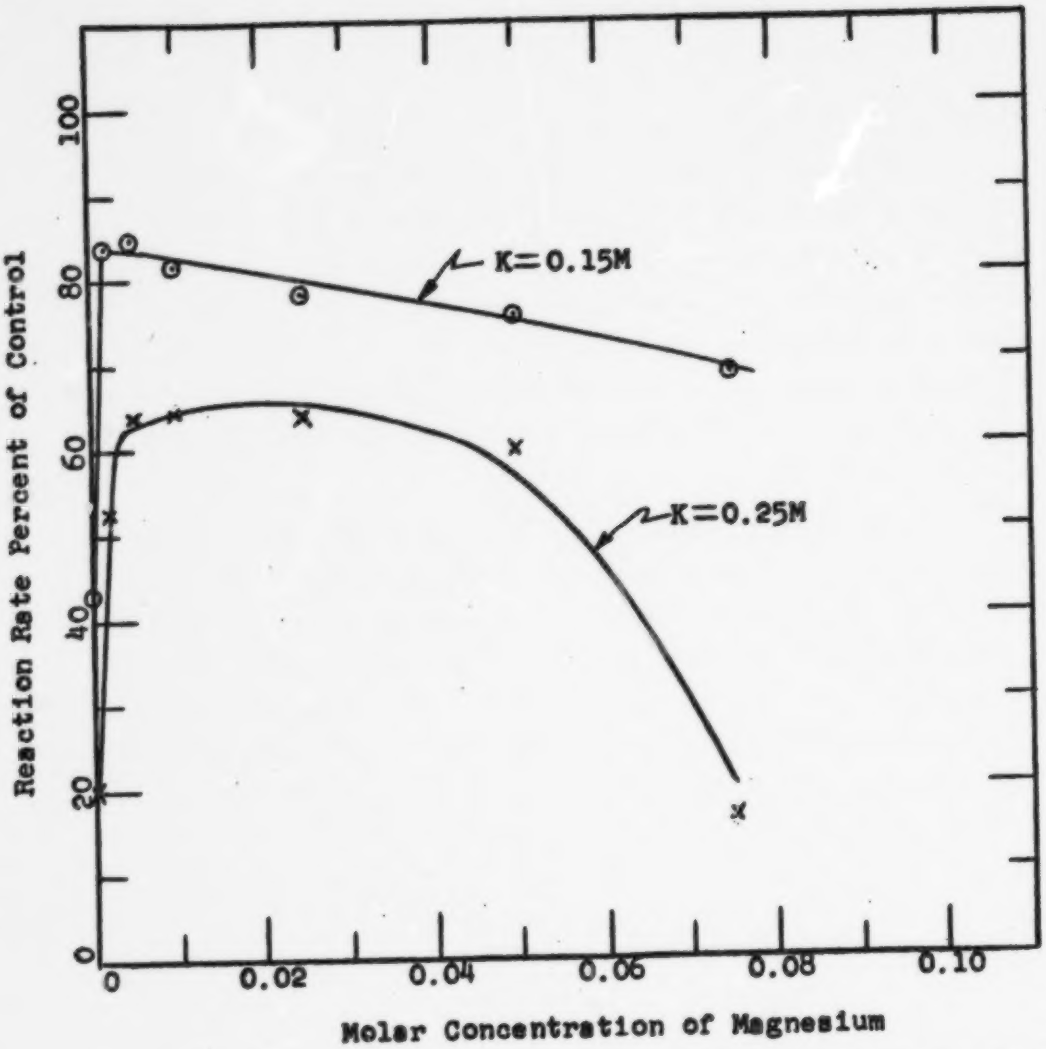


FIGURE 19 - EFFECT OF MAGNESIUM ON POTASSIUM INHIBITION

At 0.25M potassium the peak was not obtained until the magnesium concentration reached 5×10^{-3}M. The peak range for antagonism was 5×10^{3}M to 0.05M. However, at this concentration of potassium a very rapid fall in antagonism beyond the peak range was obtained with a cross over occurring at about 0.07M magnesium.

Ammonium

The effect of ammonium on potassium toxicity is shown in Figure 20. These results show a pattern of antagonism somewhat at variance with that observed in all other antagonistic relationships. At both levels of potassium, antagonism increased with ammonium concentration. At high levels of ammonium the amount of antagonism approached a constant value. The range of ammonium concentrations where the constant antagonism was reached was 0.05M to 0.1M. The antagonism achieved with ammonium was less than that achieved with the other ions tested.

It was noted in Run 3 that the plot of gas production versus time for units with high concentrations of potassium showed a definite change in reaction rate after about half the gas production was complete. This effect was also observed in all studies on antagonism of potassium inhibition. However, except for ammonium this effect was completely abolished by the presence of any of the antagonists used even when the concentration of antagonist was beyond the cross over concentration. When ammonium served as the antagonist, this change in reaction rate was still observed at low concentrations of ammonium. As the concentration of ammonium increased, this change in rate effect was gradually eliminated. Complete elimination was achieved at 0.05M ammonium.

Runs 19 and 20 - Effect of Sodium on Magnesium Inhibition

In these two runs the magnesium concentration was raised to 0.075M, 0.1M, 0.125M, and 0.2M in mixed liquor from the magnesium acclimation unit. The range of sodium concentrations used was 0 to 0.1M.

The results are shown in Figure 21. These results show that sodium is an excellent antagonist of magnesium. In fact at levels of 0.075M and 0.1M magnesium, in the range of sodium concentrations which yielded maximum antagonism, reaction rates greater than the control were obtained. At 0.075M magnesium the antagonism peak range is from 5×10^{-3}M to 0.035M sodium. In this range

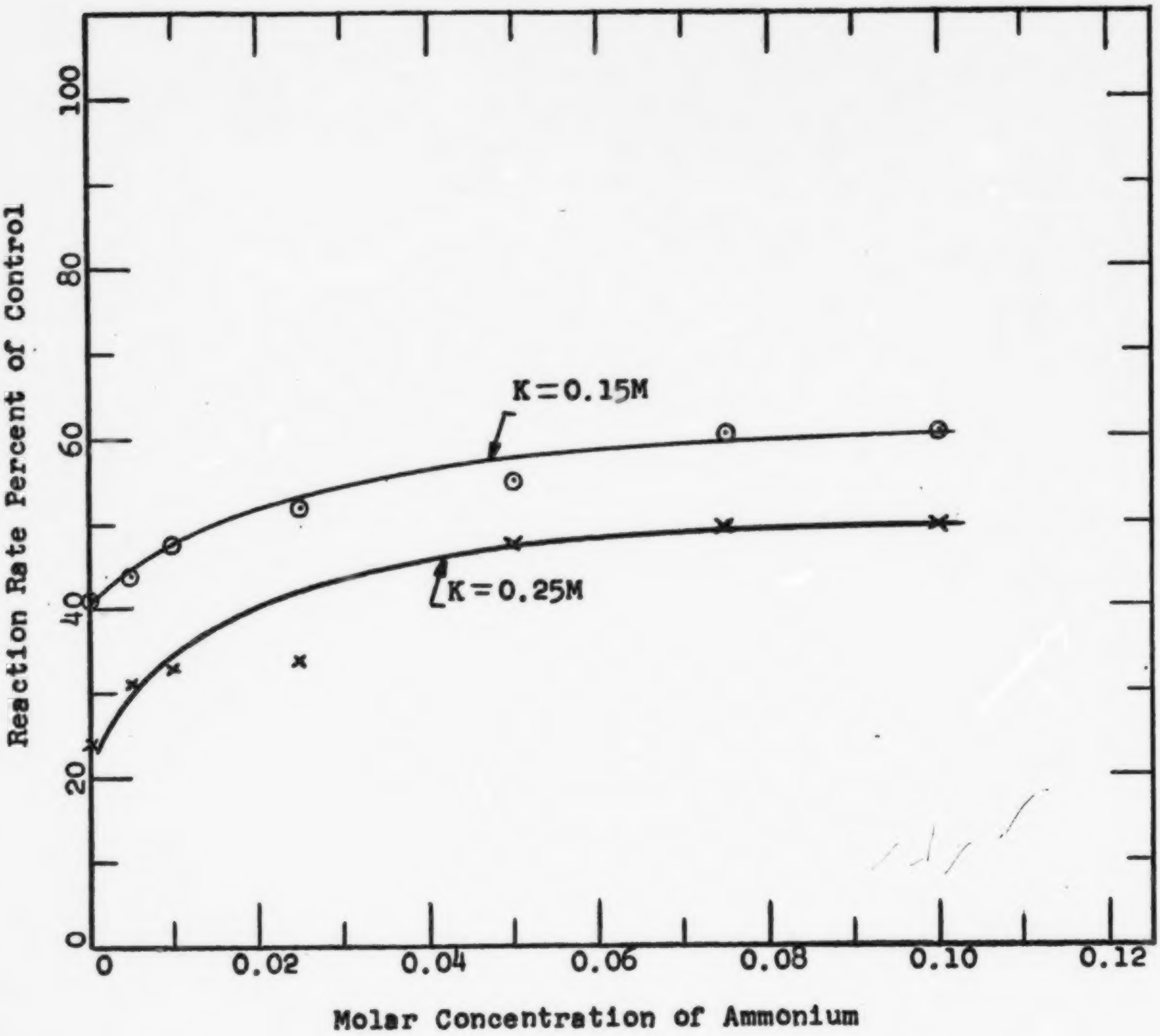


FIGURE 20 - EFFECT OF AMMONIUM ON POTASSIUM INHIBITION

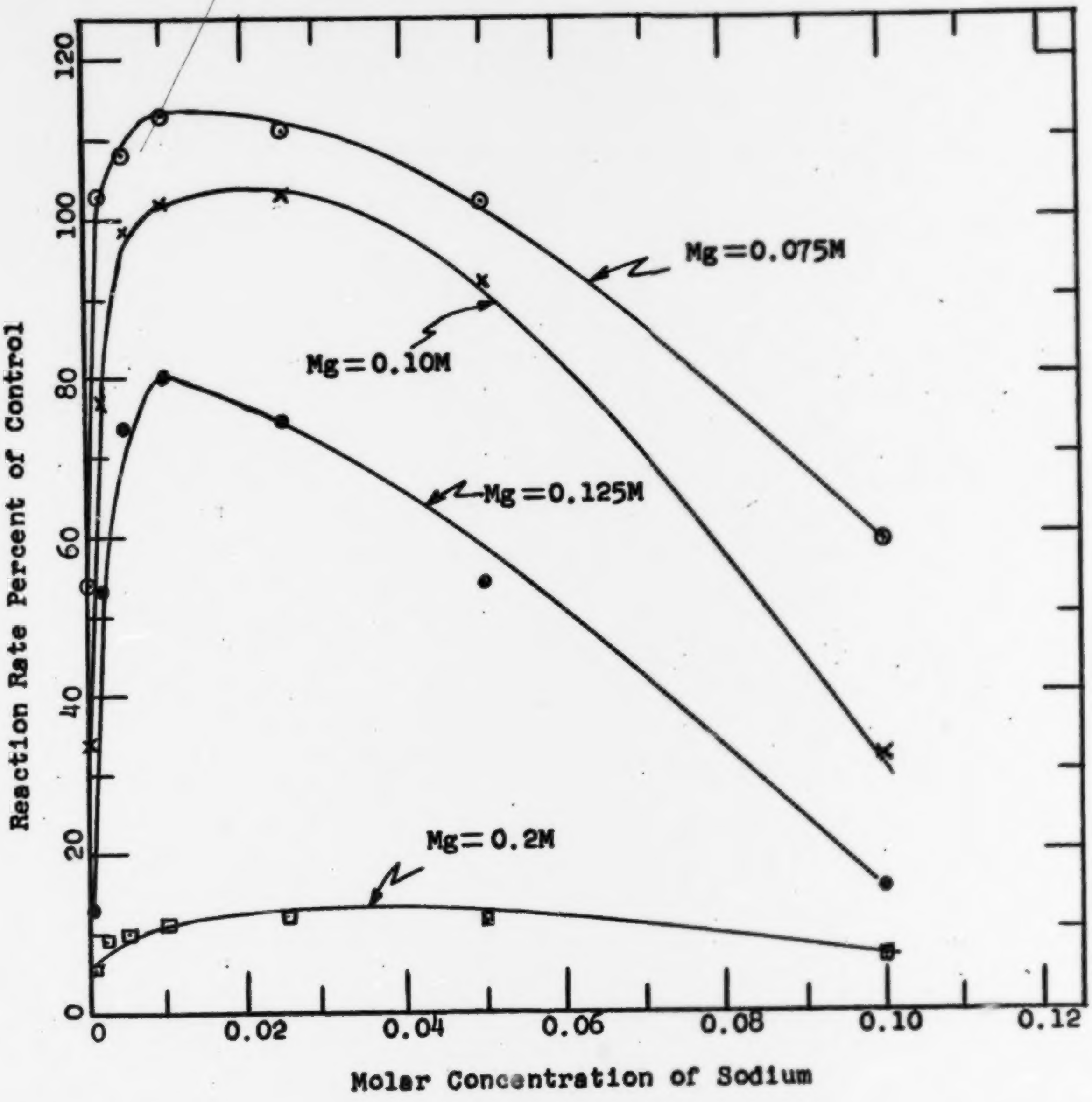


FIGURE 21 - EFFECT OF SODIUM CONCENTRATION ON MAGNESIUM INHIBITION

the reaction rate was 110 percent of the control. Even the very low sodium concentration of 2 x 10^{-3}M produced a slight stimulation over the control rate. As the sodium concentration increased past the peak range, the antagonism decreased markedly. At 0.1M sodium the reaction rate was only slightly greater than that for the unit containing no added sodium.

At 0.1M magnesium the same pattern of antagonism was observed. The range of peak antagonism was slightly narrowed over that obtained with 0.075M magnesium. In particular the antagonism achieved with 2 x 10^{-3}M sodium was considerably less with respect to the peak antagonism than was obtained when the magnesium concentration was 0.075M. Again the antagonism achieved fell off considerably as the sodium concentration increased past the peak range. A crossover was observed at 0.1M sodium.

At 0.12M magnesium the same pattern was repeated. The range of peak antagonism again narrowed and the crossover point was again found to occur near 0.1M sodium. At 0.2M magnesium a very slight antagonism was observed. The magnitude of antagonism at this value of magnesium is too slight to be considered significant.

Runs 21 & 22 - Effect of Potassium on Magnesium Inhibition

The procedures used for these runs were the same as those for Runs 19 and 20. The range of potassium used was 0 to 0.12M.

The results are shown in Figure 22. Potassium is shown to be a good antagonist of magnesium by these results, but the antagonism achieved is inferior to that achieved with sodium. On the other hand the fall-off of antagonism with increasing potassium concentration is considerably less than when sodium is the antagonist. This same effect was noted in Runs 10 and 11. While potassium could not antagonize ammonium and sodium could, high levels of potassium in combination with ammonium were less inhibitive than high levels of sodium in combination with ammonium.

In general the results obtained with potassium are very similar to those with sodium as the antagonist. At the lowest concentration of magnesium (0.075M) the range of peak antagonism was from 2 x 10^{-3}M to 60 x 10^{-3}M. At 0.1M magnesium the peak antagonistic range narrowed to 5 x 10^{-3}M to 60 x 10^{-3}M. The range of peak antagonism further narrowed to 5 x 10^{-3}M to 45 x 10^{-3}M at 0.125M magnesium. As with sodium, no antagonism was achieved at 0.2M magnesium.

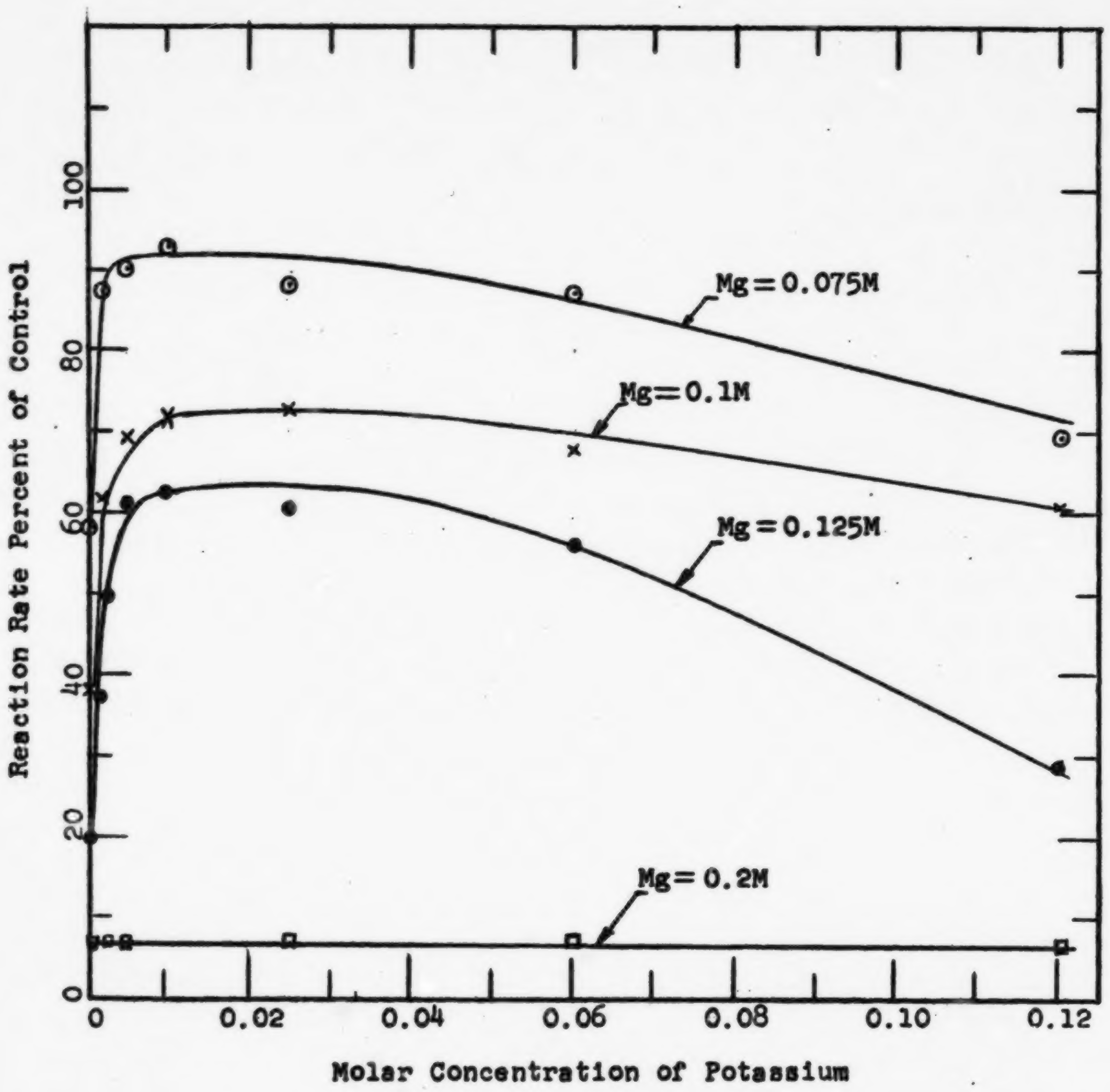


FIGURE 22 - EFFECT OF POTASSIUM CONCENTRATION ON MAGNESIUM INHIBITION

Run 23 - Effect of Ammonium on Magnesium Inhibition

The levels of magnesium used in this study were 0.075M and 0.125M. The concentrations of ammonium were 0 to 0.1M.

The results of this run are shown in Figure 23. These results show that unlike the other monovalent ions sodium and potassium, ammonium is not an antagonist of magnesium. The presence of ammonium slightly increases the apparent inhibition of magnesium. At both levels of magnesium, an increase of inhibition is observed up to 0.025M ammonium. Increasing concentrations beyond this value of ammonium appear to exert little extra inhibitive effect although the general trend is downward.

Run 24 - Effect of Calcium on Magnesium Inhibition

The procedures and purpose were the same as Run 23. The calcium level used was in the range 0 to 0.1M.

The results are given in Figure 24. The results indicate that mixtures of magnesium and calcium are more inhibitive than either alone. At 0.075M magnesium the addition of calcium quickly results in a fall in the rate of reaction. The fall-off starts at 0.01M calcium, a non-toxic concentration of calcium when present alone. The reaction rate falls off quickly with calcium concentration until a concentration of 0.05M calcium. The fall-off levels off at calcium concentrations greater than 0.05M, reaching 10 percent of the control at 0.075M magnesium plus 0.1M calcium.

At the higher magnesium level a slow fall-off is observed with increasing calcium concentration with a leveling out at concentrations greater than 0.05M calcium. Increased inhibition also starts to become evident at 0.01M calcium at this magnesium level. It is interesting to note that the rate of reaction for a mixture of 0.075M magnesium and 0.05M calcium is equal to that for 0.125M magnesium.

Run 25 - Effect of Sodium and Potassium on Calcium Inhibition

In this study the ability of sodium and potassium to individually antagonize calcium inhibition was investigated. Only one inhibitory level of calcium 0.1M was utilized. Attempts to run studies at higher calcium levels were hindered by the sudden release of carbon dioxide at higher calcium concentrations. This was due to the precipitation of calcium carbonate. The values of sodium and potassium used were 0 to 0.1M and 0 to 0.12M, respectively.

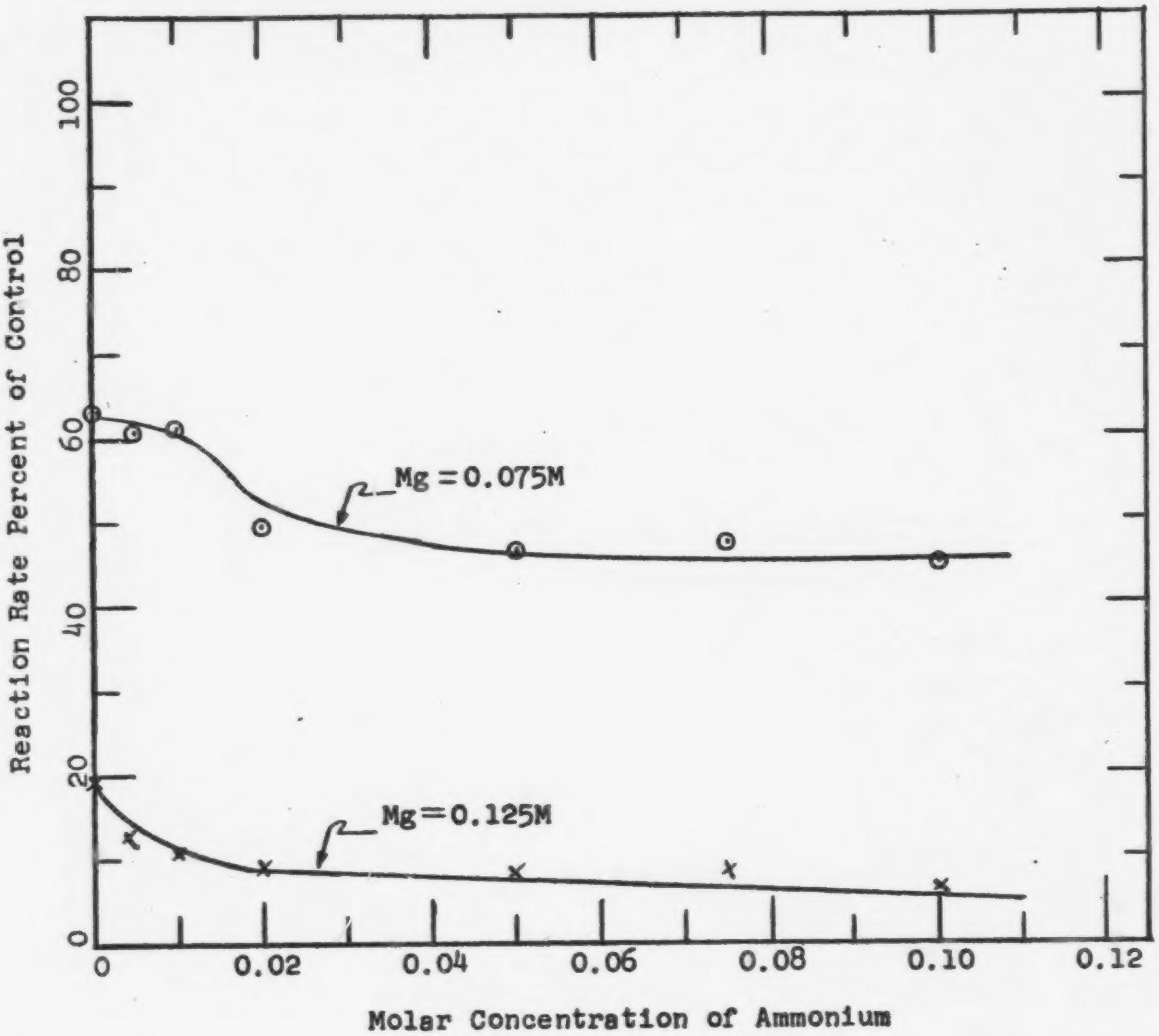


FIGURE 23 - EFFECT OF AMMONIUM ON MAGNESIUM INHIBITION

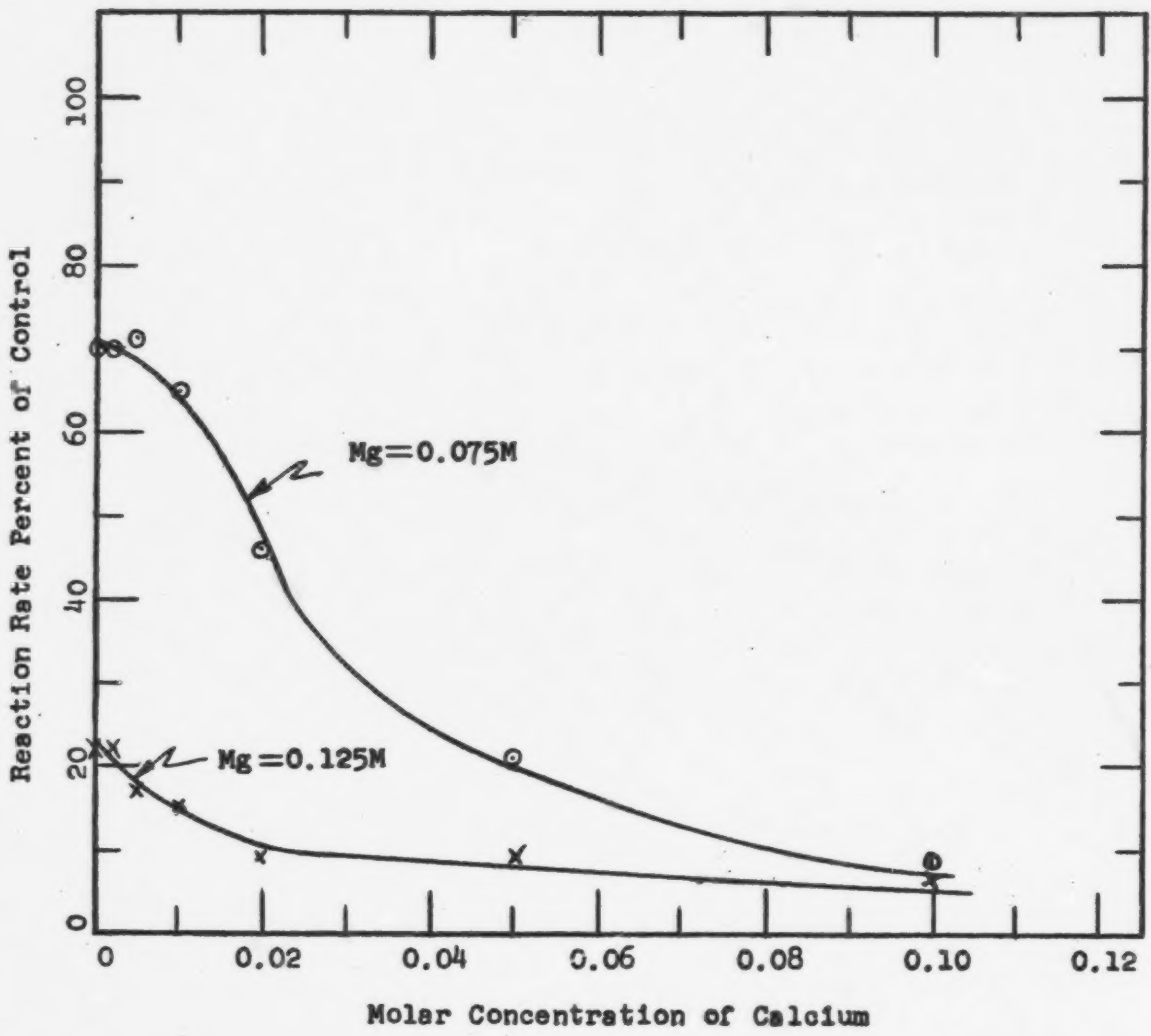


FIGURE 24 - EFFECT OF CALCIUM ON MAGNESIUM INHIBITION

Sodium

The results are shown in Figure 25. These results show that the pattern observed for sodium antagonism of calcium is similar to that for sodium antagonism of magnesium. At low concentrations of sodium, very good antagonism is achieved. Peak antagonism occurs between 5×10^{-3}M to 25×10^{-3}M sodium. As the concentration of sodium increases beyond the peak, a very rapid fall-off in antagonism is evident. The crossover point is reached at 0.05M sodium.

Potassium

These results are presented in Figure 26. Here also the results obtained are very similar to those obtained for potassium antagonism of magnesium. Good antagonism is achieved at low concentrations of potassium, but not as good as with sodium as the antagonist. The peak range of antagonism is 2×10^{-3}M to 60×10^{-3}M potassium. At potassium concentrations past the peak, a slow decrease in antagonism is observed. The crossover concentration occurs at approximately 0.11M potassium.

Run 26 - Effect of Magnesium and Ammonium on Calcium Inhibition

In this run the same procedures were utilized as in Run 25. The ranges of magnesium and ammonium used were 0 to 0.075M and 0 to 0.1M, respectively.

Ammonium

The results are shown in Figure 27. The results here are again similar to those observed for the effect of ammonium on magnesium toxicity. As ammonium increases beyond 1×10^{-2}M, the apparent inhibition of calcium significantly increases. This increase tends to level out at concentrations beyond 0.05M ammonium. The increase in toxicity observed is greater here than was observed when combinations of ammonium and magnesium were used (Run 23).

Magnesium

The results are shown in Figure 28. Again the results are similar to those observed for combinations of calcium and magnesium in Run 24. Additions of magnesium above 5×10^{-3}M magnesium produced significant increases in the inhibition of calcium. It appears from the results in Run 24 and this run that the toxicity of a system containing calcium and magnesium is very close to that obtained in a system containing only one of these ions in a concentration equal to the sum of the two.

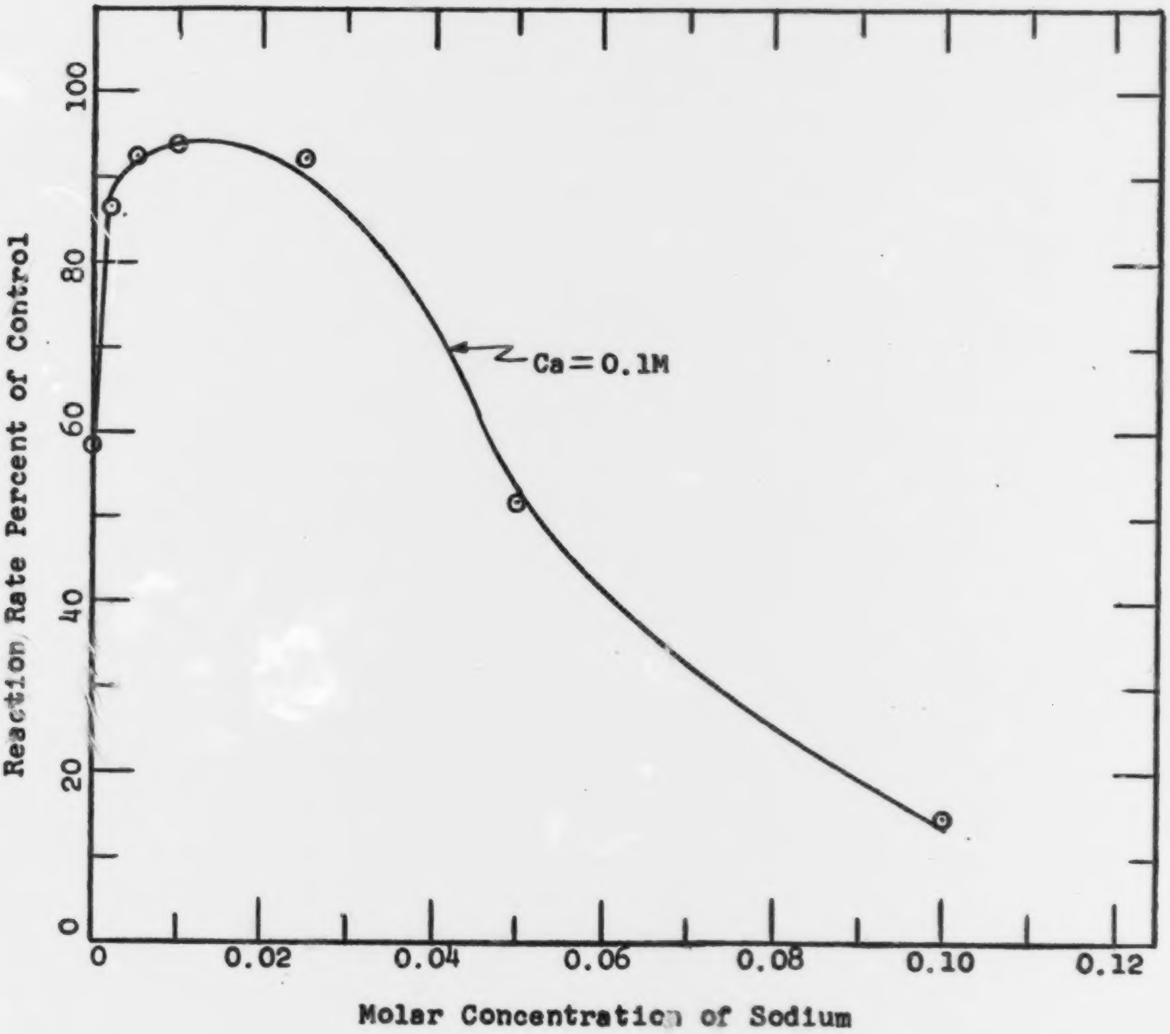


FIGURE 25 - EFFECT OF SODIUM CONCENTRATION ON CALCIUM INHIBITION

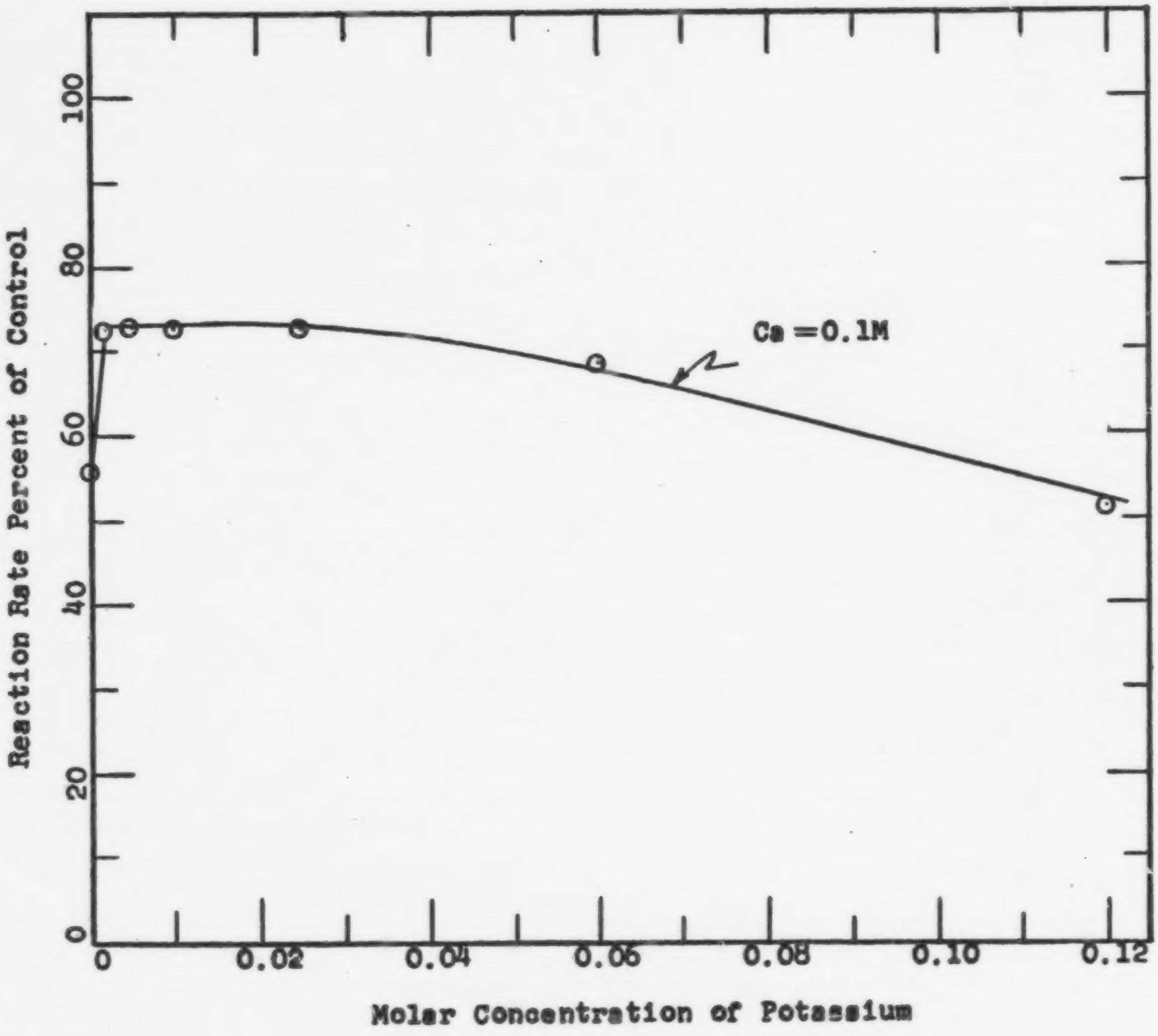


FIGURE 26 - EFFECT OF POTASSIUM CONCENTRATION ON CALCIUM INHIBITION

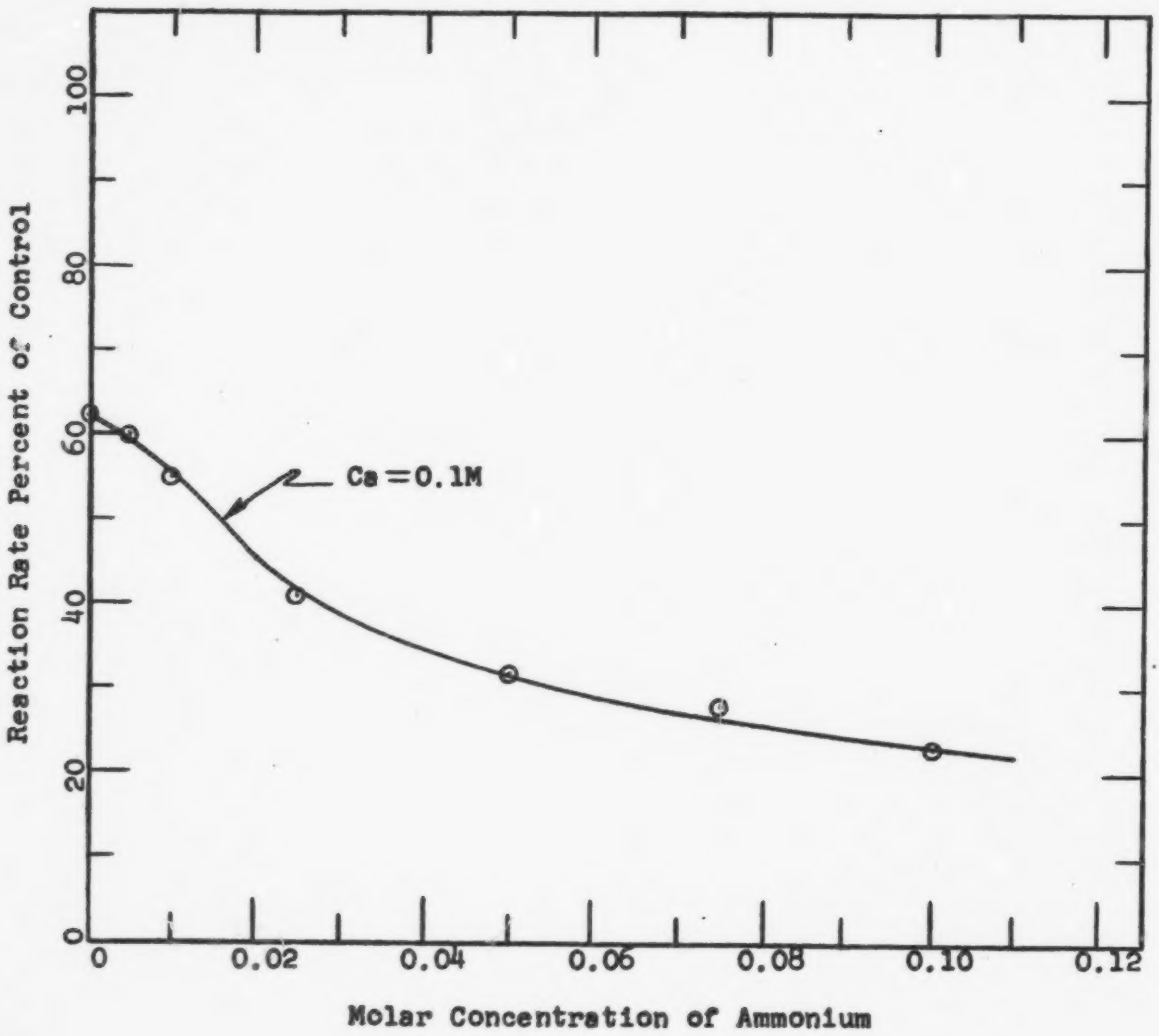


FIGURE 27 - EFFECT OF AMMONIUM ON CALCIUM INHIBITION

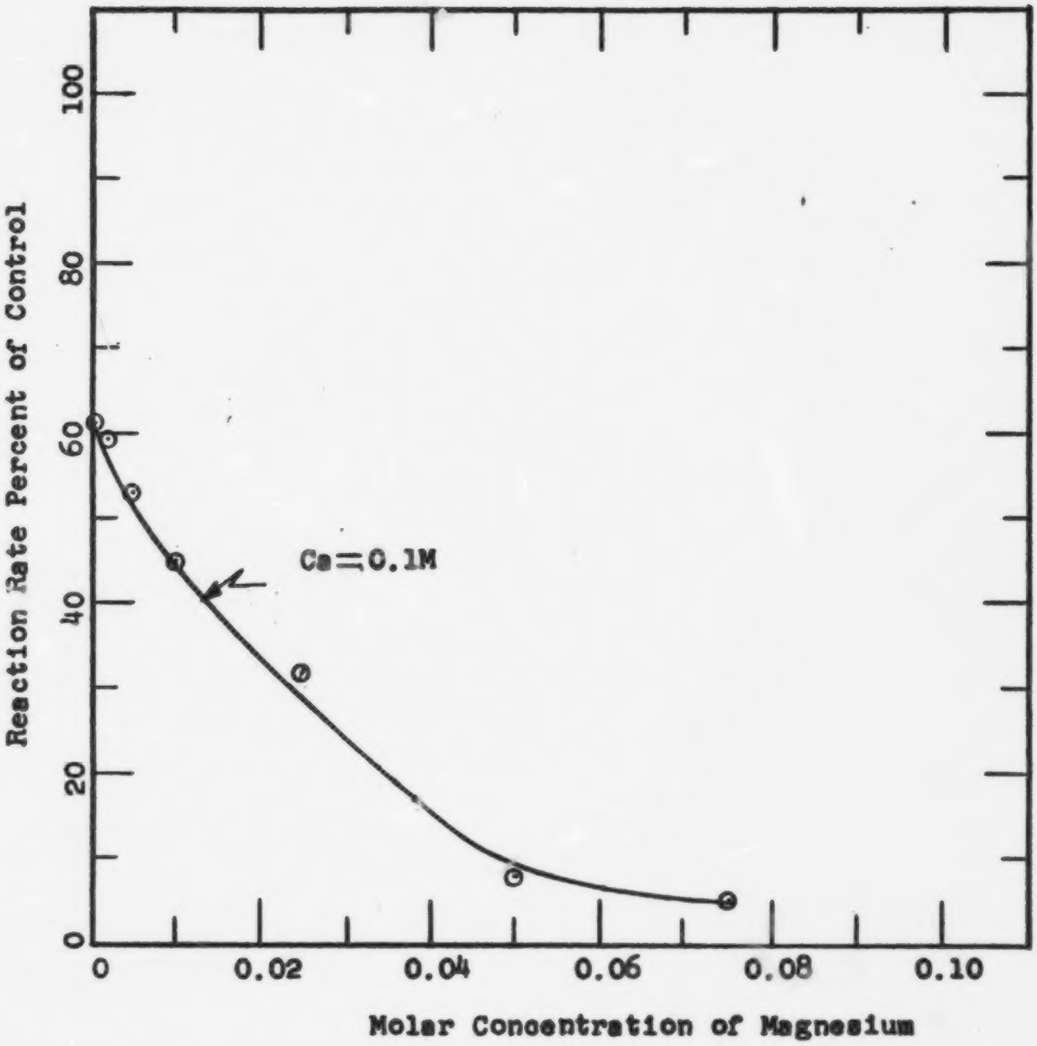


FIGURE 28 - EFFECT OF MAGNESIUM ON CALCIUM INHIBITION

Run 27 - Antagonism of Sodium Inhibition by Combinations of Cations

The results of Runs 6 - 9 indicated that in a dual cation system, only potassium could antagonize sodium inhibition. In this run the ability of various ions to antagonize sodium inhibition in combination with potassium was investigated One value of sodium concentration was used, 0.45M. In addition, only one concentration of each ion was used. The concentrations used were 0.01M potassium, 0.01M ammonium, 0.005M calcium, and 0.005M magnesium. The concentrations of these potential antagonists was kept low as the previous studies have shown that antagonism is most effective at low concentrations of the antagonists.

The results of this run are shown in Figure 29. These results indicate that the combination of potassium and calcium produced the best results. This combination raised the reaction rate to 42 percent of the control, while the presence of potassium alone produced a reaction rate of 29.5 percent of the control. The combination of magnesium plus potassium produced only a very slight increase over potassium. The combination of potassium and ammonium produced results identical to the units containing only potassium as the antagonist.

The addition of magnesium, and a combination of magnesium and ammonium to the potassium-calcium combination produced no extra antagonism above that produced by the potassium-calcium combination. Nor did the addition of these combinations reduce the amount of antagonism achieved by the potassium-sodium combination. Excellent agreement was achieved between duplicates in the run.

These results indicate that the effects noted for the multiple ion antagonism observed are due to the individual characteristics of the ions at the concentration present. The extra increment of antagonism produced by the presence of calcium cannot be due to a secondary antagonism of potassium, the primary antagonist, because (1) potassium is not toxic at this low concentration, and (2) magnesium, which is a better antagonist of potassium than calcium, did not produce any appreciable increase in antagonism over the system containing potassium alone.

Run 28 - Antagonism of Ammonium Inhibition by Combinations of Cations

The results of Runs 10 - 13 indicated that in a dual cation system, only sodium could antagonize ammonium inhibition. In this run various combinations of potassium, calcium, and magnesium, each cation at 0.005M concentration, were tested with 0.01M and 0.02M sodium, as to their ability to antagonize ammonium inhibition. In all units except the control, the ammonium ion concentration was maintained at 0.25M.

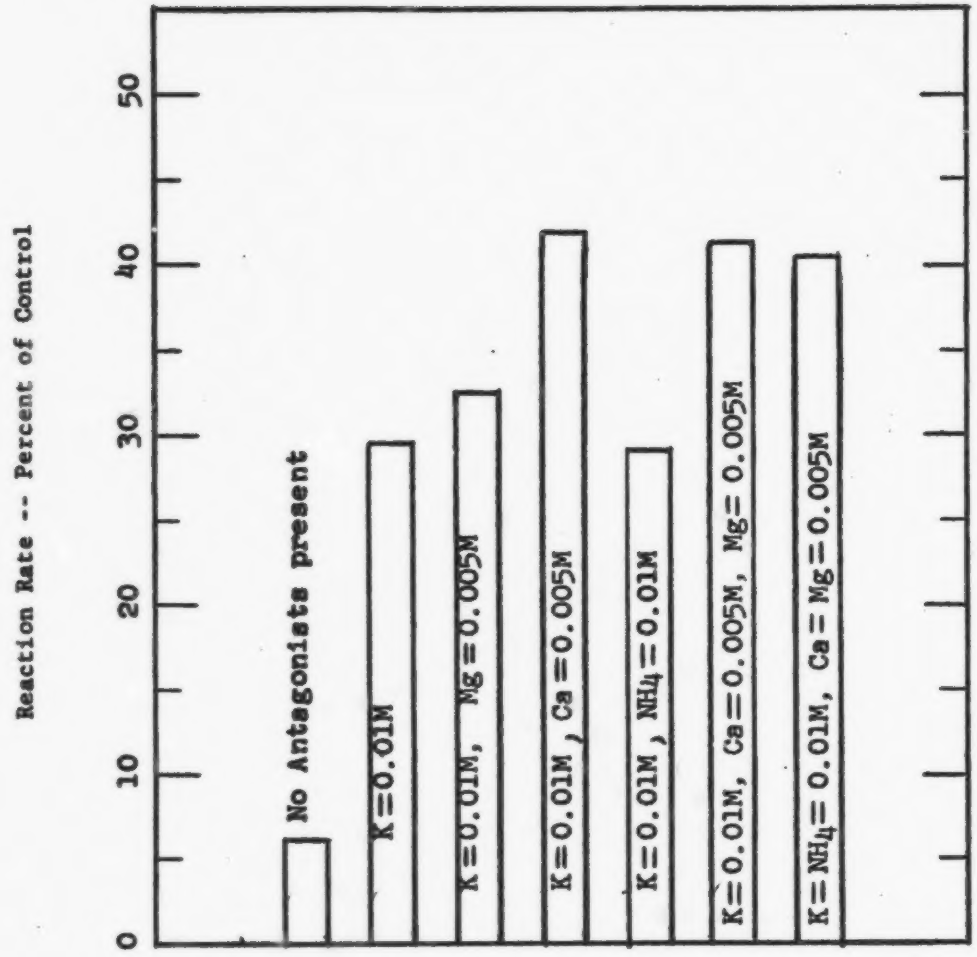


FIGURE 29 - ANTAGONISM OF SODIUM BY A COMBINATION OF CATIONS

The results of this run are shown in Figure 30. These results again indicate that a combination of cations can produce antagonism superior to that achieved by a single antagonist. Combinations of sodium plus potassium, and sodium plus magnesium produce a slight increase over the antagonism produced by sodium alone. The multiple cation combinations used produced even better antagonism than combinations of two antagonists.

These findings support the findings of Run 27. Cations that do not function as antagonists when they are present in a dual cation system with the inhibitor, can increase the antagonism achieved by a primary antagonist.

Run 29 - Antagonism of Potassium Inhibition by Combinations of Cations

In this run various combinations of 0.01M sodium, 0.01M ammonium, 0.005M calcium, and 0.005M magnesium were tested as to their ability to antagonize 0.3M potassium.

The results of this run are shown in Figure 31. These results are summarized below:

(1) Combinations which include sodium plus calcium in the ions present produce best antagonism.

(2) The presence of magnesium and/or ammonium slightly decrease the antagonism produced by sodium.

(3) Calcium, magnesium, and ammonium either singly or in combination produce about the same antagonism.

(4) The presence of magnesium and/or ammonium does not reduce the antagonism produced by combinations of sodium plus calcium.

(5) At this level of potassium, the antagonism produced by sodium is slightly greater than that produced by calcium or magnesium.

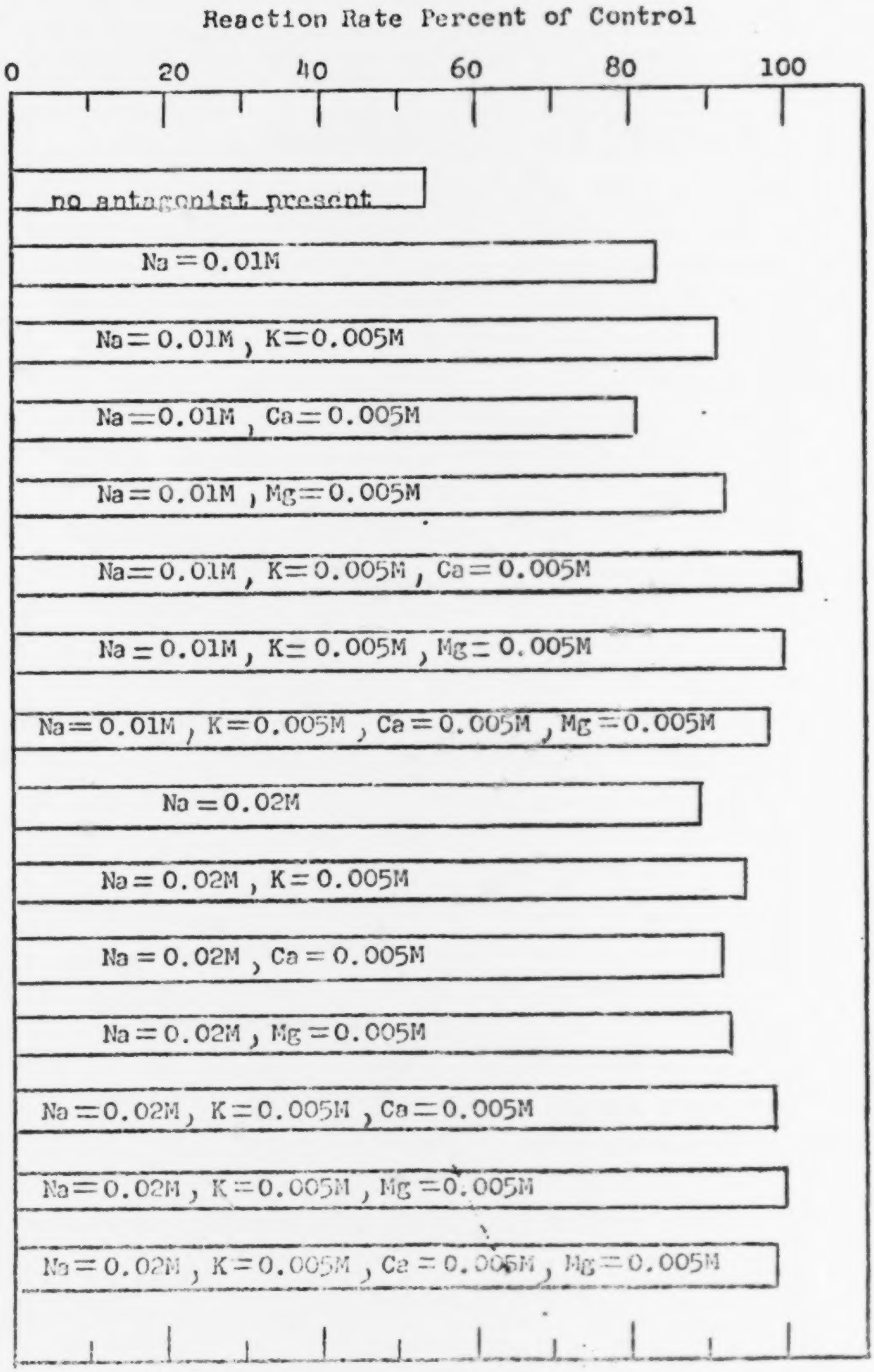
These results again show that combinations of cations can produce antagonistic action superior to that of single cations.

Run 30 - Antagonism of Magnesium Inhibition by Combinations of Cations

In this run the ability of combinations of 0.01M sodium, 0.01M potassium, 0.01M ammonium, and 0.005M calcium to antagonize the inhibition of 0.125M magnesium was investigated.

The results of this run are shown in Figure 32. These results are summarized as follows:

FIGURE 30 - ANTAGONISM OF AMMONIUM BY COMBINATIONS OF CATIONS



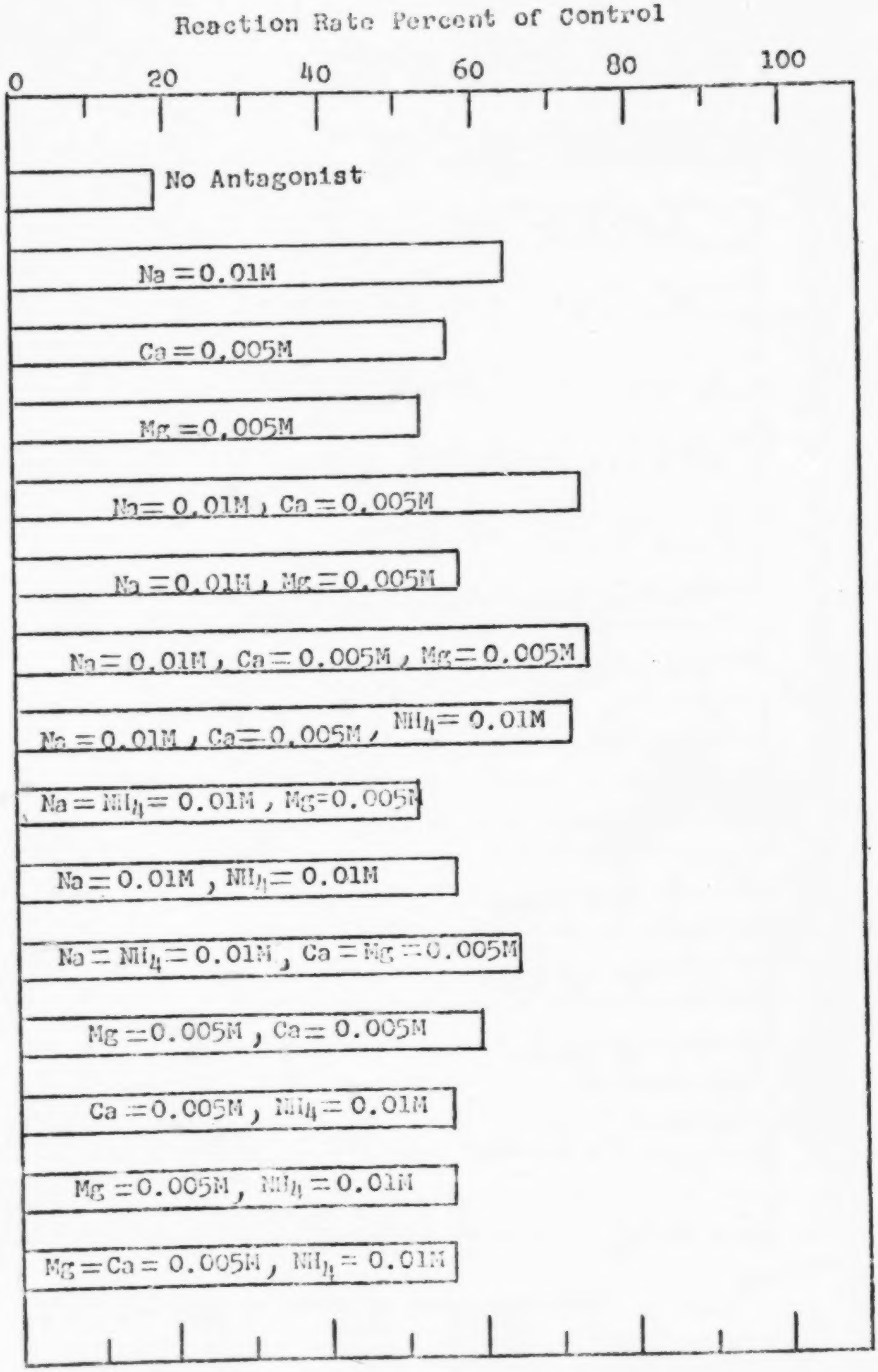


FIGURE 31 - ANTAGONISM OF POTASSIUM BY COMBINATIONS OF CATIONS

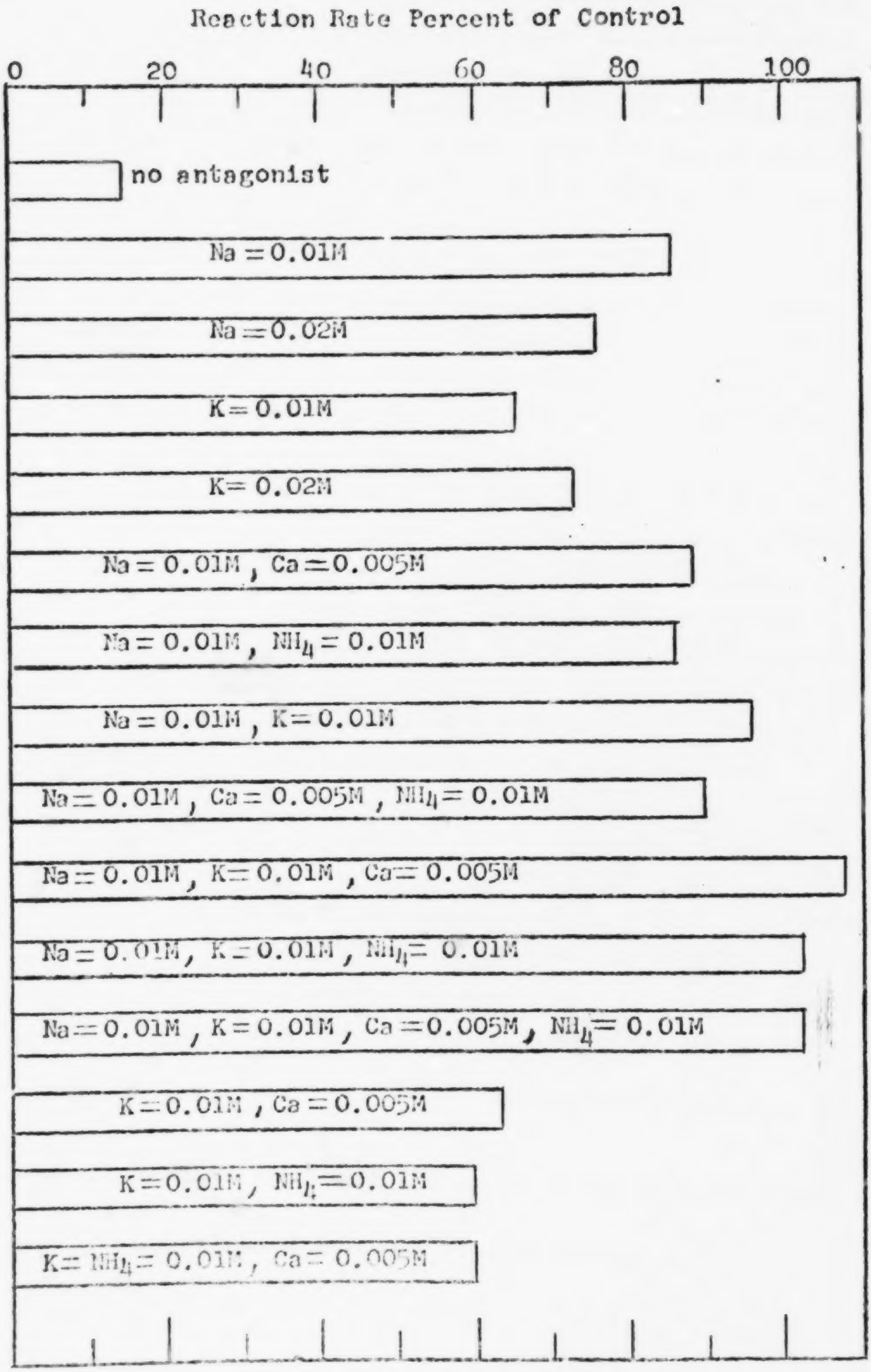


FIGURE 32 - ANTAGONISM OF MAGNESIUM BY COMBINATIONS OF IONS

(1) Neither calcium nor ammonium, alone nor in combination, can improve the antagonism produced by sodium or potassium alone.

(2) Combinations of sodium and potassium produce antagonism greater than that produced by either ion alone.

(3) This extra antagonism is not due to just the presence of more monovalent cation, because a combination of 0.01M sodium plus 0.01M potassium produces better results than either 0.02M sodium or 0.02M potassium alone.

(4) Both calcium and ammonium, singly and in combination, increase the antagonism produced by combinations of sodium and potassium.

(5) The best results are obtained with a combination of sodium, potassium, and calcium.

(6) Several combinations produced reaction rates superior to the control rate.

D. Results of Daily Feed Studies

The first four daily feed studies, Runs 1 to 4, had a two-fold purpose:

(1) To determine whether and to what extent acclimation would take place if the salt concentration was slowly increased.

(2) To determine whether the antagonistic relationships observed in the slug feed studies would hold as well in daily feed studies.

These studies were all conducted on a batch feed basis at a 15-day detention time. Not all of the antagonistic relationships observed in slug feed Runs 6 to 30 were tested in these runs, because sufficient space was not available in the Incubation Room. No studies were conducted on ammonium toxicity because a satisfactorily operating set of units with an ammonium buffer could not be established.

The last daily feed study, Run 5, was concerned with the nature of sodium inhibition and the effect of this inhibition on bacterial growth.

Run 1 - Sodium Inhibition, Daily Feed

Five units were utilized in this study, with one serving as a control. The sodium concentration was raised in the other four units by raising the concentration of sodium chloride in the feed to a constant value for a period of several days. At two times during the study the sodiumchloride concentration in the feed was increased. The sodium concentrations in the feed during this study are given in Table 3. The antagonistic ions were raised to appropriate concentrations in the units on the first day of the study and maintained at this concentration throughout the study. In Table 4 the ions fed to the various units in this study are presented.

Table 3

Concentration of Sodium in the Feed at Various Times During Run 1

Sodium Concentration	Time During Study
0.3M	Day 1 - 16
0.4M	Day 17 - 30
0.5M	Day 31 - 33

Table 4

Salts Fed to Units During Run 1

Unit	Salts
1	Control
2	NaCl
3	NaCl + 0.01M KCl
4	NaCl + 0.01M NH_4Cl
5	NaCl + 0.01M KCl + 0.01M NH_4Cl

The operation of these units was followed by daily determinations of the gas production and volatile acid concentration in the effluent. The effluent volatile acids plotted versus the number of days after the start of the run are shown in Figure 33. An alternate scale showing the sodium concentration in the units is also shown in this figure.

These results show a slow increase in sodium inhibition as the sodium concentration increases beyond 0.2M. At the end of the study the sodium concentration had increased to 0.35M, but the unit containing only sodium was still operating well. At this concentration the efficiency of operation based on acetic acid removal was 84 percent. In the slug feed studies (Run 1), this concentration of sodium reduced the reaction rate to 25 percent of the control. Thus, definite acclimation is evident.

The antagonistic relationships observed in this study are in line with those observed in the slug feed studies (Runs 6 - 9). The unit containing 0.01M potassium showed only a very slight inhibition which first became evident at 0.275M sodium. At the end of the run the efficiency of operation of this unit was 92 percent, while the efficiency of the control was 94.5 percent. The unit containing 0.01M ammonium showed no antagonism of sodium which is in agreement with the results of slug feed Run 7. Neither did the combination of potassium and ammonium produce any increase in the antagonism produced by potassium, which is agreement with results of slug feed Run 27.

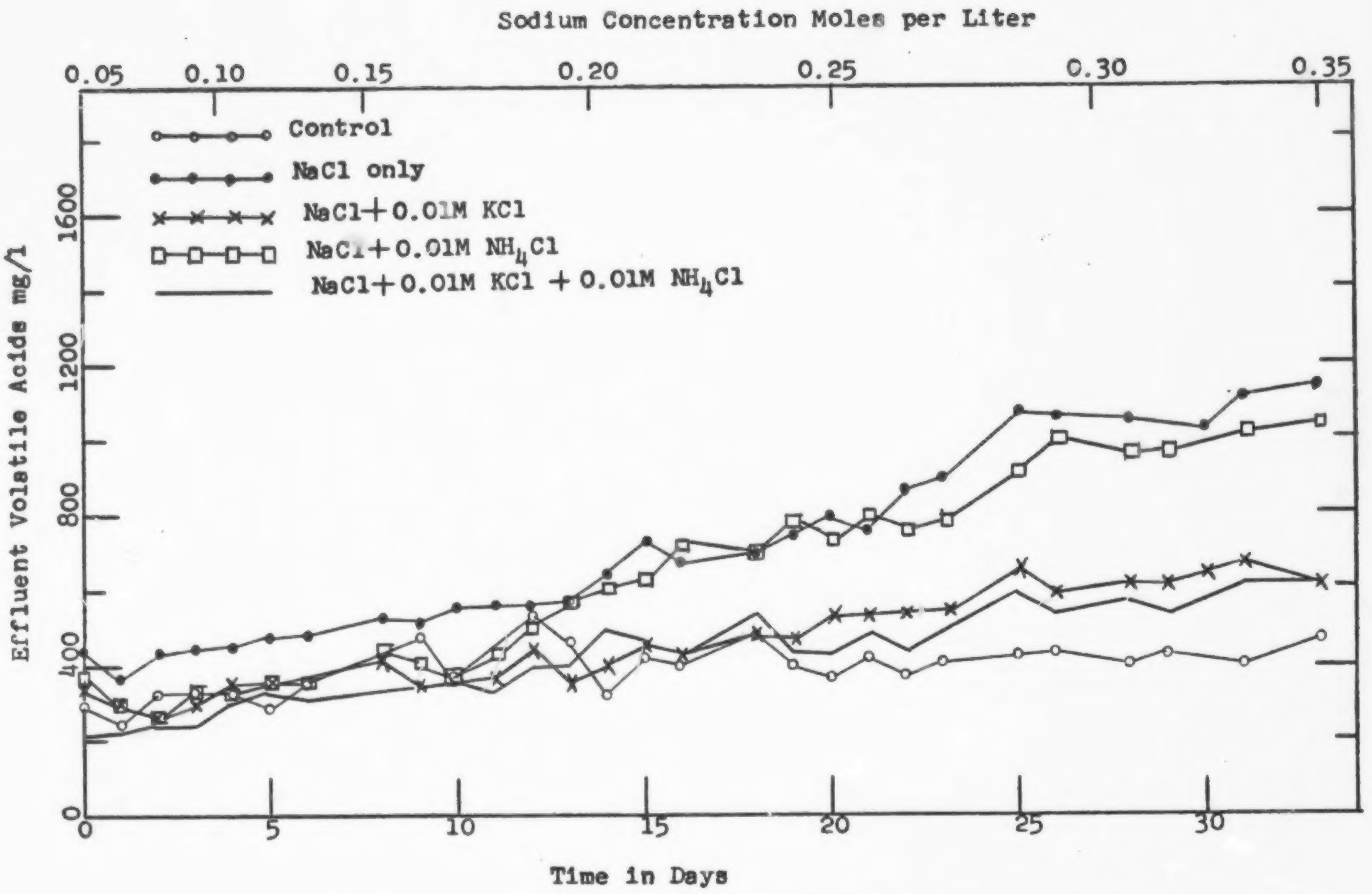


FIGURE 33 - OPERATION OF DAILY FEED UNITS DURING RUN 31

Run 2 - Potassium Inhibition, Daily Feed

The procedures used in this run were similar to those used in Run 1 except for the ions and concentrations used. A control with a buffer of 2500 mg/l of potassium bicarbonate as calcium carbonate was employed. The ions and concentrations used are shown in Tables 5 and 6.

Table 5

Concentration of Potassium in the Feed in Run 2

Potassium Concentration	Time During Run
0.3M	Day 1 - 20
0.4M	Day 21 - 28
0.5M	Day 29 - 33

Table 6

Salts Fed to the Units in Run 2

Unit	Salts
1	KCl
2	Control
3	KCl + 0.01M NaCl
4	KCl + 0.005M $CaCl_2$
5	KCl + 0.01M NaCl + 0.005M $CaCl_2$

The volatile acid data plotted versus time from the start of the run is shown in Figure 34. A scale showing the potassium concentration present is also shown in this figure.

These results show that no inhibition was exhibited by potassium until the concentration reached 0.13M. Beyond this value the inhibition increased rapidly until death occurred at about 0.23M potassium. Thus, acclimation is again evidenced as on a slug feed basis (Run 4), a value of 0.13M potassium reduced the reaction rate to 60 percent of the control. The acclimation achieved in this run was not as good as that in Run 1. Perhaps this was due to the fact that rate of build-up to an inhibitive concentration was faster in this run than in Run 1, so that the organisms were given less of a chance to acclimate.

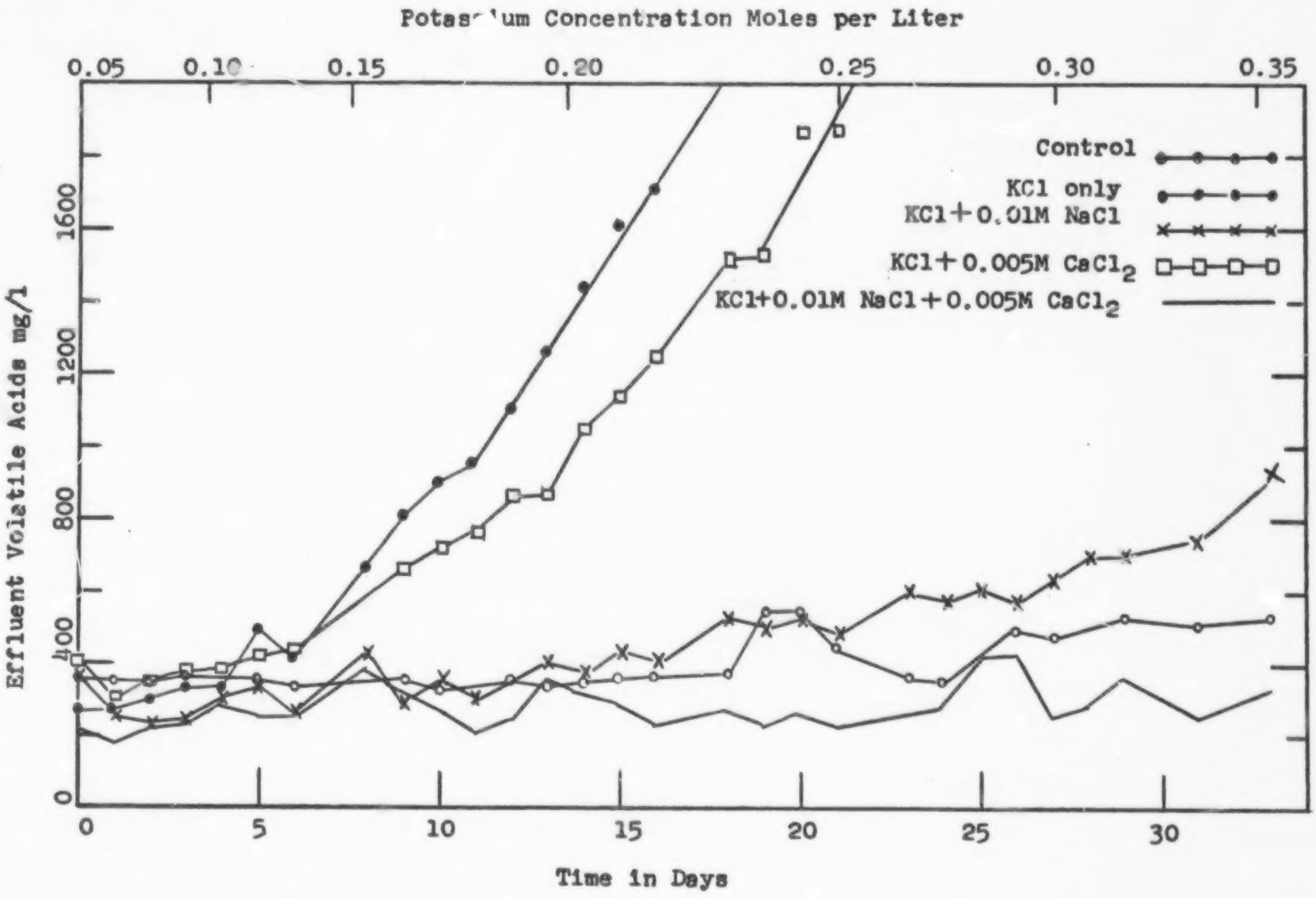


FIGURE 34 - OPERATION OF DAILY FEED UNITS - RUN 32

The antagonistic relationships observed in this study compare favorably with those observed in the slug feed runs. Both sodium and calcium were able to antagonize the potassium inhibition. In the slug feed runs sodium produced only slightly better antagonism than calcium. In this study, however, sodium produced much better antagonism than calcium. With calcium as the antagonist, inhibition began at the same potassium concentration as when o calcium was present. However, the rate of increase of inhibition beyond this point was lower than when no calcium was present.

Excellent antagonism was achieved with sodium as the antagonist. Inhibition did not begin until the potassium concentration reached 0.3M. Even at 0.35M potassium the operation of this unit was quite satisfactory as it had an efficiency of operation of 89 percent.

In confirmation of the results obtained in slug feed Run 29 the antagonism achieved with a combination of calcium and potassium was superior to that achieved with either of these antagonists. In fact the results achieved were so good that even at a potassium concentration of 0.35M the operation of this unit was superior to that of the control. The efficiency of operation of the control was 93.5 percent, while that in the unit containing calcium and sodium as antagonists was 96 percent.

Run 3 - Magnesium Inhibition, Daily Feed

The procedures used in this run were the same as in Runs 1 and 2 except for the ions studied. The magnesium concentration in the feed solution during the run is shown in Table 7. The salts fed to the various units are shown in Table 8.

Table 7

Concentration of Magnesium in the Feed in Run 3

Magnesium Concentration	Time During Run
0.1M	Day 1 - 16
0.15M	Day 17 - 30
0.20M	Day 31 - 33

Table 8

Salts Fed to Units in Run 3

Unit	Salt
1	Control
2	$MgCl_2$ only
3	$MgCl_2$ + 0.01M KCl
4	$MgCl_2$ + 0.01M NaCl
5	$MgCl_2$ + 0.01M KCl + 0.01M NaCl

The volatile acid data are plotted in Figure 5. These data again show that acclimation will take place if the salt concentration is slowly raised. Magnesium did not exhibit inhibition until the concentration reached 0.06M. This concentration produced a reaction rate of 70 percent of the control on a slug feed basis. At concentrations greater than 0.06M the toxicity increased until the unit showed complete failure at 0.1M. The failures noted in this run and in Run 2 were not because the salt concentration reduced the rate of bacterial action to zero. Rather, the rate slowed down to such an extent that the volatile acids exceeded the buffer capacity and a low pH was actually responsible for the final failure.

Again in this run, antagonisms similar to those observed in the slug feed runs were obtained. Potassium gave good antagonism delaying the onset of appreciable inhibition till about 0.09M magnesium, and complete failure till 0.13M magnesium. Sodium produced even better results -- inhibition was delayed until about 0.11M magnesium. At the highest concentration of magnesium used (0.14M) the unit containing 0.01M sodium had an operational efficiency of 86.5 percent. For practical purposes no significant inhibition had appeared at this concentration.

The combination of sodium and potassium produced even better results than sodium or potassium alone which is in agreement with the results of slug feed Run 30.

Run 4 - Calcium Inhibition, Daily Feed

The calcium concentrations in the feed during this run is shown in Table 9. The salts fed to the various units during the run are shown in Table 10.

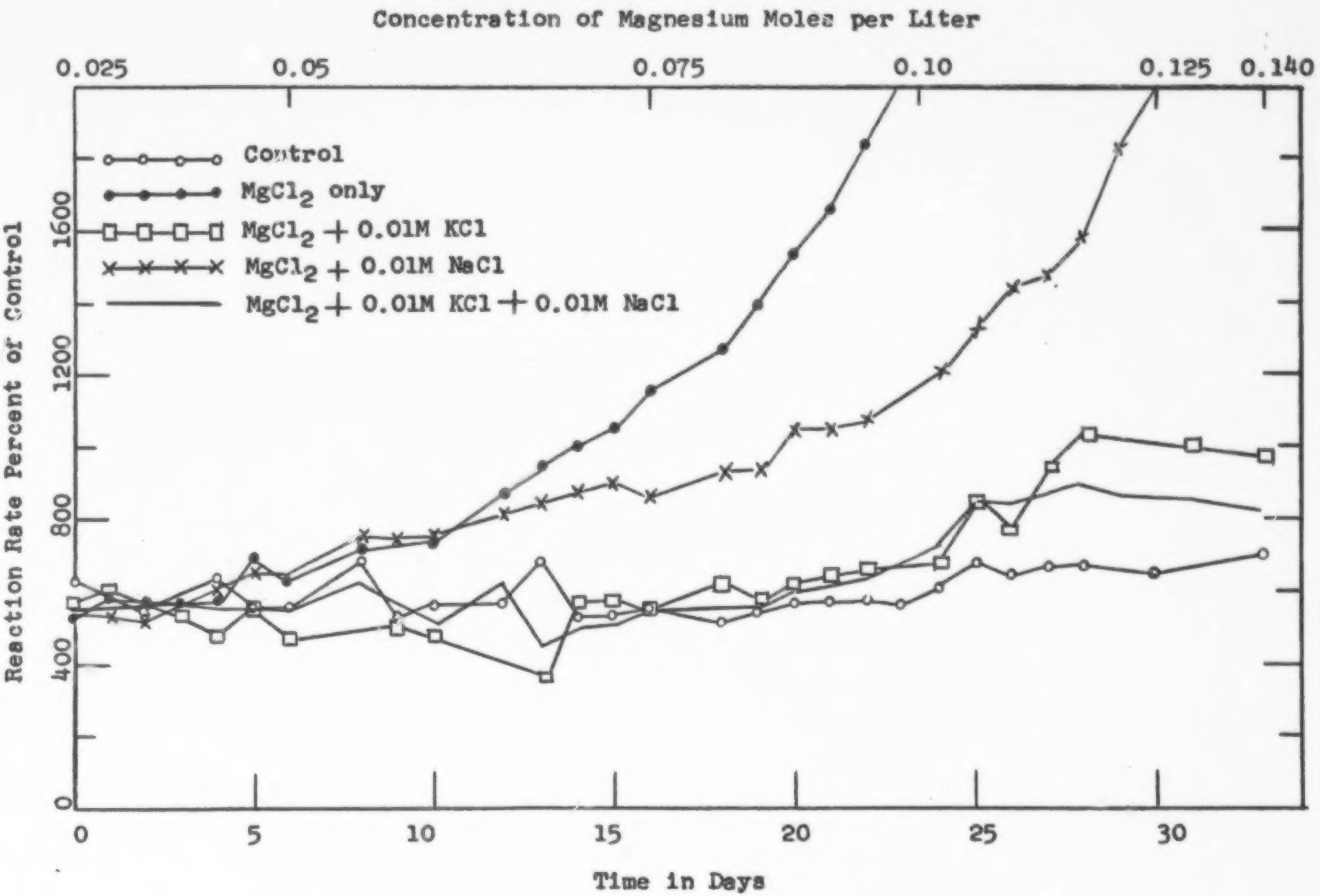


FIGURE 35 - OPERATION OF DAILY FEED UNITS DURING RUN 33

Table 9

Calcium Concentration in the Feed During Run 4

Calcium Concentration	Time During Run
0.15M	Day 1 - 15
0.20M	Day 16 - 27
0.30M	Day 28 - 31

Table 10

Salts Fed to the Units During Run 4

Unit	Salts
1	Control
2	$CaCl_2$
3	$CaCl_2$ + 0.01M NaCl
4	$CaCl_2$ + 0.01M NaCl + 0.005M KCl
5	$CaCl_2$ + 0.01M NaCl + 0.005M $MgCl_2$

The volatile acid data are plotted in Figure 36. These data show that a very significant amount of acclimation to calcium inhibition occurred in this run. For all practical purposes no significant inhibition was produced by calcium in this run. A slight increase in inhibition was observed at 0.17M calcium, but the operation of the unit was still quite satisfactory at 0.2M calcium. On a slug feed basis (Run 5) calcium produced absolute inhibition at a concentration of 0.2M. It is probable that a considerable portion of the calcium supposedly in solution actually precipitated as calcium carbonate in this run. It was observed that the sides of the digesters were covered with a white precipitate. Thus, the acclimation achieved in this run may be more apparent than real. It is also probable that some of the inhibition of calcium on a slug basis was due to a pH shock. Undoubtedly this factor can also be acclimated to over a period of time.

Significant antagonism was achieved by the sodium ion in this run. In fact, all units containing sodium either alone or in combination with other ions ran better than the control. The units containing both combinations of magnesium plus sodium, and potassium plus sodium showed slightly better results than sodium alone. A slug feed run on antagonism of calcium was not performed, but these results are in line with what would be expected in view of the results from the other studies on antagonism by combinations of ions.

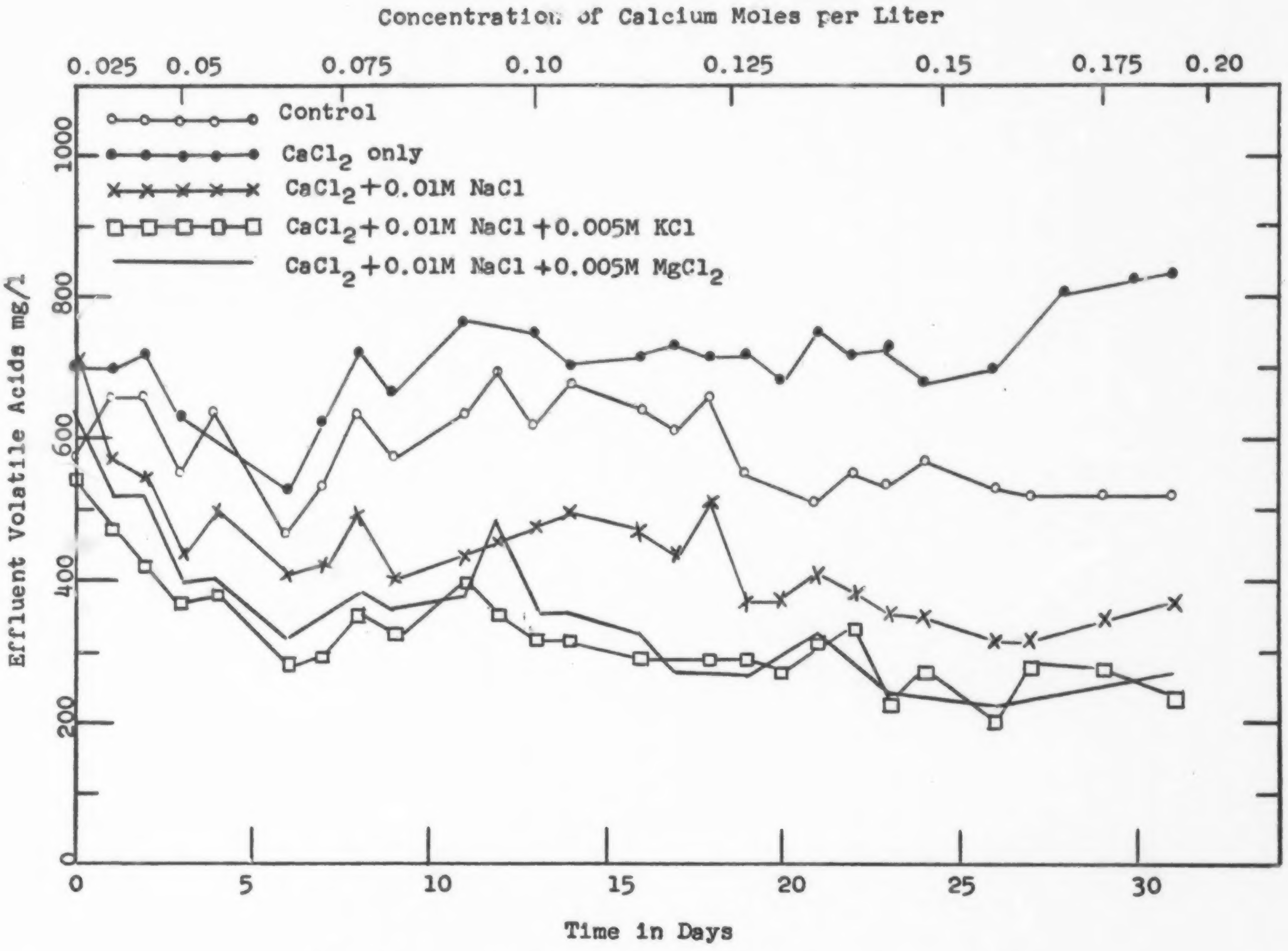


FIGURE 36 - OPERATION OF DAILY FEED UNITS - RUN 34

Run 5 - Effect of Sodium on Bacterial Synthesis

In this run five units with a sodium buffer were established at a feed rate of one gram per liter per day of acetic acid, at a 15-day detention time. One unit served as a control while the sodium concentration in the feed to the other units was maintained at 2, 3, 4, and 6 grams per liter (0.087M, 0.13M, 0.174M, 0.26M respectively). The operation of the units was checked by periodic effluent volatile acid determinations. Organic nitrogen was periodically run on these units to determine the synthesis of bacterial protoplasm taking place.

The volatile acid data is shown plotted against the time from the beginning of the run in Figure 37. With the type of sodium addition used in this run the rate of increase of sodium decreased with time; because with time the concentration in the unit approached that in the feed. For all practical purposes after three detention times (45 days), it can be assumed that the concentration in the unit equaled that in the feed. The results show an increase in the volatile acid concentration in the units during the early part of the run with a leveling out once the sodium concentration was stabilized. The higher the sodium concentration, the lower the efficiency of operation attained. Even the low concentration of two grams per liter of sodium produced a slight but definite decrease in efficiency.

The 6 g/l sodium unit did not show any tendency for the efficiency to level out, but showed a continual slow increase in effluent volatile acid concentration. It is possible that a leveling out would have eventually occurred, as this unit was furthest from its theoretical sodium concentration of all the units. Despite the high effluent volatile acids from this unit, the operation was apparently satisfactory as the lowest operational efficiency attained was 90 percent.

On the 53rd day 0.011M potassium was added to the 6 g/l unit. This produced an immediate increase in efficiency (from 90 to 95 percent) which was maintained for the remainder of the study. On the 85th day 0.0085M potassium was added to the four g/l unit. This addition also produced an immediate increase in the efficiency of operation. This again indicates that antagonism will take place even when acclimation to cation inhibition is evident.

In Figures 38 - 41 plots of operational efficiency versus time and ratio of organic nitrogen in the unit to that in the control versus time are presented for each unit. These plots show that the organic nitrogen ratio

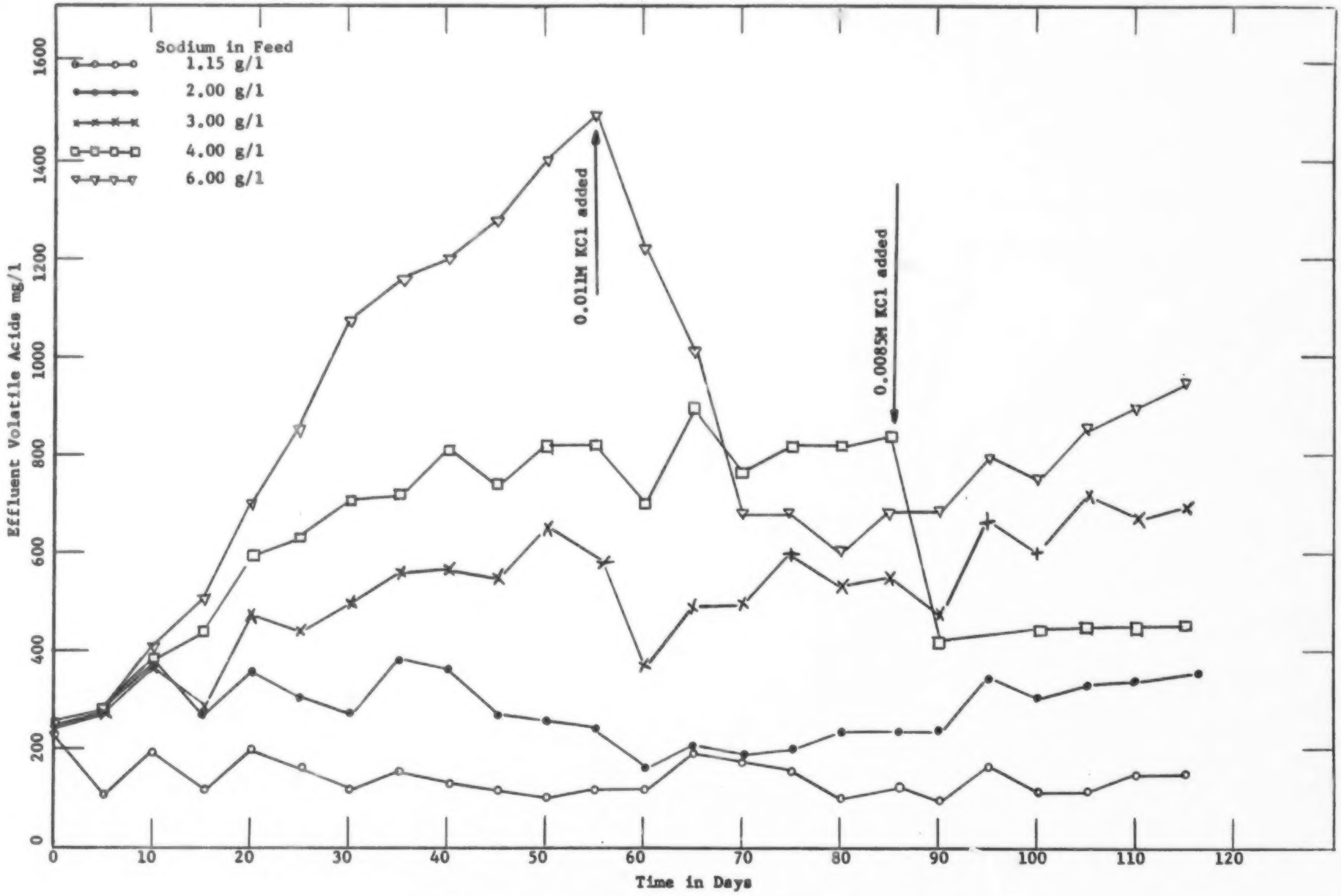


Figure 37 - OPERATION OF DAILY FEED UNITS DURING RUN 35

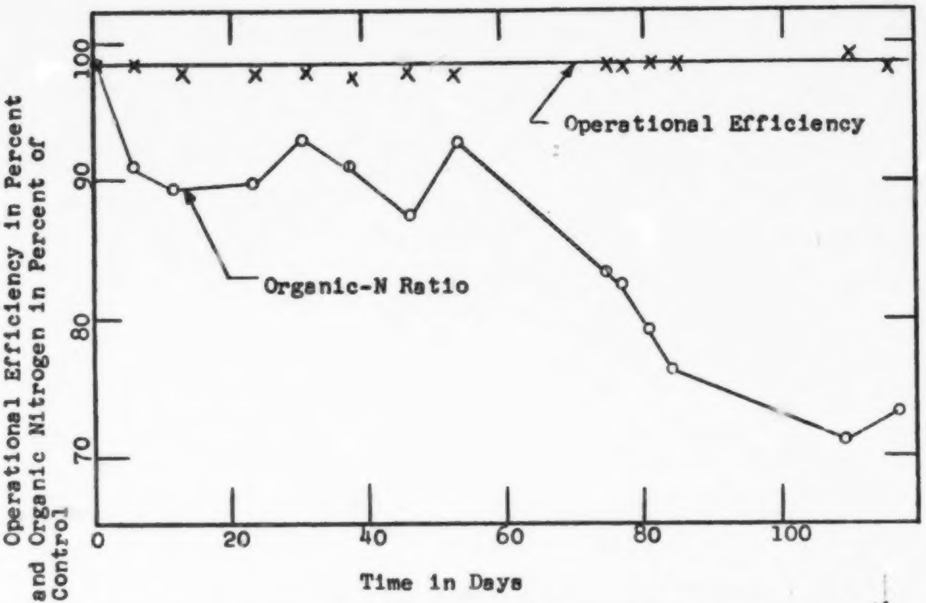


FIGURE 38 - ORGANIC NITROGEN AND OPERATIONAL EFFICIENCY OF THE UNIT CONTAINING 2000 MG/L OF SODIUM DURING RUN 35

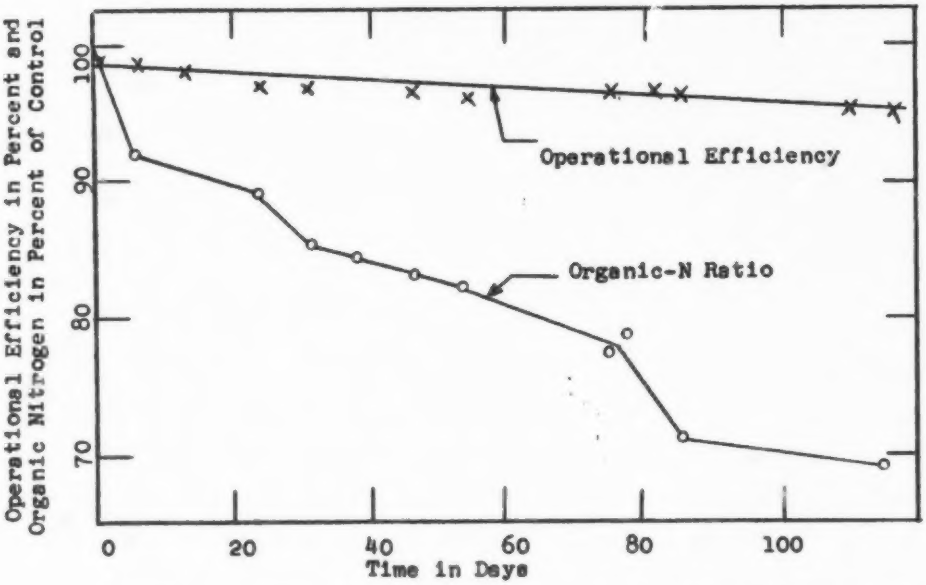


FIGURE 39 - ORGANIC NITROGEN AND OPERATIONAL EFFICIENCY OF THE UNIT CONTAINING 3000 MG/L OF SODIUM DURING RUN 35

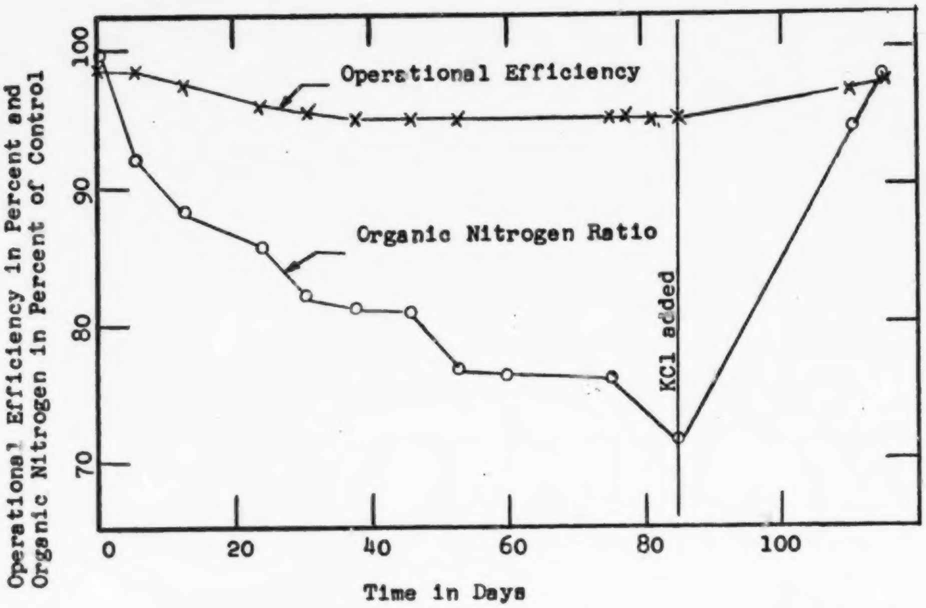


FIGURE 40 - ORGANIC NITROGEN AND OPERATIONAL EFFICIENCY OF THE UNIT CONTAINING 4000 MG/L OF SODIUM DURING RUN 35

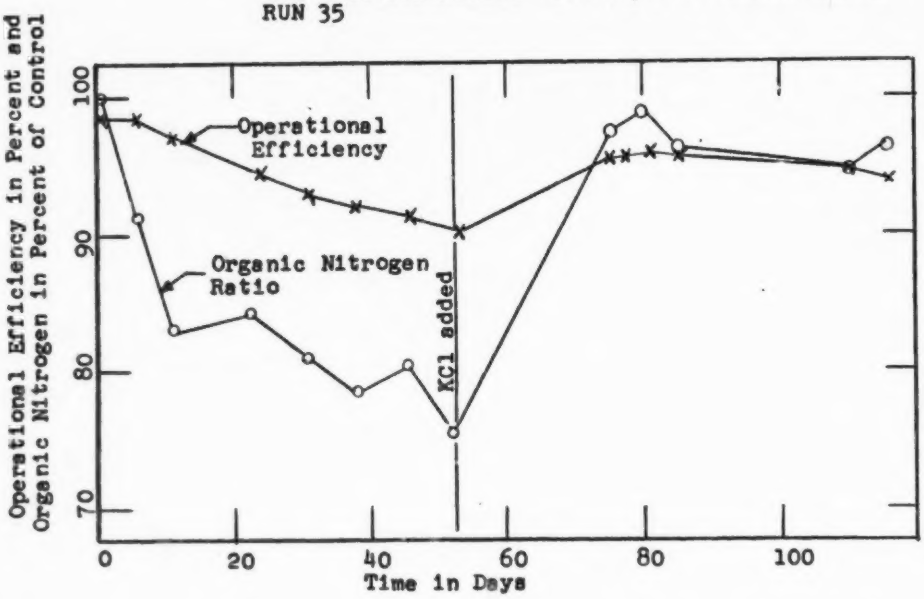


FIGURE 41 - ORGANIC NITROGEN AND OPERATIONAL EFFICIENCY OF THE UNIT CONTAINING 6000 MG/L OF SODIUM DURING RUN 35

decreased to a greater extent in each unit than the efficiency of operation. The greater the sodium concentration, the greater the disparity between the efficiency and organic nitrogen values. This indicates that the presence of sodium at concentrations below those that will produce a marked inhibitive effect interfere with the synthesis of bacterial protoplasm. The best example of this is the two g/l sodium unit. Although this unit operated at an efficiency greater than 98 percent throughout the run, the organic nitrogen in this unit had fallen to 73 percent of the control by the end of the run.

The addition of potassium to the four and six g/l units produced a complete alleviation of this synthesis block, although it did not completely antagonize the inhibitive effect of sodium on the substrate utilization. By the end of the run the efficiency of operation of each of these two units was very close to the organic nitrogen ratios with respect to the control.

The operational efficiency and organic nitrogen ratios used in plotting Figures 38 - 41 are given in Table 11.

Table 11

Efficiency of Operation and Organic Nitrogen Ratios for the Units in Run 5

	Operational Efficiency %					Ratio of Organic-N in the Unit to the Organic-N in the Control			
Time in Days	Control	Na 2 g/l	Na 3 g/l	Na 4 g/l	Na 6 g/l	Na 2 g/l	Na 3 g/l	Na 4 g/l	Na 6 g/l
0	98.5	98.5	98.5	98.5	98.5	98.5	100	101	100
6	99.5	98.5	98.5	98.5	98.5	91.0	92.0	92.0	91.0
13	99.5	98.0	98.0	97.5	97.0	89.5	---	88.0	83.0
24	99.0	98.0	97.0	96.0	94.5	90.0	89.0	85.5	84.5
31	99.0	98.0	97.0	95.5	93.0	93.0	85.0	82.0	81.0
38	99.0	97.5	96.5	95.0	92.0	91.0	84.0	81.0	78.5
46	99.0	98.0	96.5	95.0	91.5	87.5	83.0	81.0	80.5
53 *	99.0	98.0	96.0	95.0	90.0	93.0	82.0	76.5	75.0
75	98.5	98.5	96.0	95.0	95.5	83.0	77.5	76.0	97.5
77	98.5	98.5	96.0	95.0	95.5	82.5	78.5	76.0	99.0
81	99.0	98.0	96.0	95.0	96.0	79.0	---	72.5	93.0
85 **	99.0	98.0	96.0	95.0	95.5	76.0	71.0	71.0	95.5
110	99.0	98.0	95.5	97.0	94.5	71.0	63.5	94.0	94.0
116	99.0	98.0	95.0	97.5	94.0	73.0	69.0	98.0	96.5

* K added to 6 g/l unit.
** K added to 4 g/l unit.

E. Discussion of Results

The four main phenomena which result from the interaction of cations with any organism are toxicity, stimulation, antagonism and nutrition. The results obtained in this study shed some light on the inter-relationships between these four phenomena. A summary of these results for dual cation systems is given in Table 12, for antagonistic relationships and Table 13 for synergistic relationships. As these inter-relationships have a decided bearing on the application of the results in practice, a short discussion of the inter-relationships will be given.

1. Cation Inter-relationships

Toxicity and Nutrition

The order of increasing toxicity found in this study was on a molar basis sodium, ammonium, potassium, calcium and magnesium. This order is that of the increasing nutritional requirements of microorganisms for these cations. Requirements for divalent cations are in the micromolar range (23)(24), and for the monovalent cations they range up to 5 mM (25)(26) except for the requirement of several marine bacteria for sodium which may reach 150 mM to 300 mM (27). Thus, the higher the nutritional requirement for a cation, the higher its concentration can be raised before inhibition results.

Nutrition and Antagonism

The concentration range for peak antagonism also follows closely the nutritional requirement range. In this study the lower range of the antagonism levels used was within the peak nutritional range of monovalent cations, but greater than that for divalent cations. Reference to Table 12 indicates that sodium was superior as an antagonist to all other cations, and that monovalent cations were superior to divalent cations as antagonists. In addition, the peak range when monovalent cations served as antagonists was broader and was not reached until a higher concentration that when divalent cations served as antagonists. The only exception to these was the lack of antagonism exhibited by ammonium. This might be explained by the fact that an excess of ammonium over the nutritional requirement was always present in the medium.

TABLE 12

ANTAGONISTIC RELATIONSHIPS IN DUAL CATION SYSTEMS

Toxic Cation	Concentration M/1	Reaction Rate % of Control With No Antagonist Present	Antagonistic Cation	Range of Peak Antagonism M/1	Reaction Rate % of Control with Peak Antagonism	Crossover Concentration M/1
Na	0.3	54	K	0.002 - 0.06	72	0.1 - 0.11
Na	0.4	30	K	0.005 - 0.03	56	0.11 - 0.12
NH_4	0.15	80	Na	0.002 - 0.05	105	0.08
NH_4	0.25	55	Na	0.005 - 0.025	67	0.045
K	0.15	40	Na	0.005 - 0.1	84	0.1
K	0.25	20	Na	0.01 - 0.05	75	0.1
K	0.35	15	Na	No Antagonism	--	--
K	0.15	41	Ca	0.002 - 0.05	78	0.1
K	0.25	20	Ca	0.005 - 0.05	59	0.1
K	0.35	16	Ca	0.002 - 0.01	38	0.05
K	0.15	43	Mg	0.002 - 0.075	85	0.075
K	0.25	20	Mg	0.005 - 0.05	64	0.07
K	0.15	41	NH_4	0.05 - 0.1	60	0.1
K	0.15	24	NH_4	0.05 - 0.1	49	0.1
Mg	0.075	54	Na	0.005 - 0.035	113	0.105
Mg	0.1	34	Na	0.005 - 0.035	103	0.1
Mg	0.125	13	Na	0.005 - 0.025	80	0.1
Mg	0.2	5	Na	No Antagonism	--	--
Mg	0.075	58	K	0.002 - 0.06	93	0.12
Mg	0.1	38	K	0.005 - 0.06	73	0.12
Mg	0.125	20	K	0.005 - 0.045	62	0.12
Mg	0.2	8	K	No Antagonism	--	--
Ca	0.1	58	Na	0.005 - 0.025	94	0.045
Ca	0.1	55	K	0.002 - 0.06	73	0.12

TABLE 13

SYNERGISTIC RELATIONSHIPS IN DUAL CATION SYSTEMS

Toxic Cation	Concentration m/1	Synergistic Cation	Concentration of Synergist Where Synergism Begins
Na	0.3	NH_4	0.025
Na	0.4	NH_4	0.01
Na	0.3	Ca	0.01
Na	0.4	Ca	0.05
Na	0.3	Mg	0.01
Na	0.4	Mg	0.05
NH_4	0.15	K	0.025
NH_4	0.25	K	0.025
NH_4	0.15	Ca	0.02
NH_4	0.25	Ca	0.01
NH_4	0.15	Mg	0.01
NH_4	0.25	Mg	0.005
Mg	0.075	NH_4	0.01
Mg	0.125	NH_4	0.005
Mg	0.075	Ca	0.005
Mg	0.125	Ca	0.005
Ca	0.1	NH_4	0.05
Ca	0.1	Mg	0.002

Antagonism and Stimulation

In several runs in the dual cation systems and in two runs involving multiple cation antagonism, stimulation above the control reaction rate was obtained. This indicates that antagonism is at least partially a function of the stimulatory nature of the cation.

Nutrition and Stimulation

It is widely held that the stimulatory action of a cation is a function of its ability to perform a nutritional function in the metabolism of the organism (10)(28).

In view of these four inter-relationships, a theory which can account for many of the results obtained in this study can be postulated. According to this theory each cation plays some nutritional role in the metabolism of an organism, the function of the cation probably being enzyme activation. As the concentration of the cation increases from zero, more and more enzyme is activated and the reaction rate increases. However, eventually all the enzyme is activated and the excess cation then reacts with an enzyme for which it is not the metallic activator. This results in a decrease in the reaction rate. Cation toxicity thus results from an excess of a nutritional factor. Antagonism is achieved by another cation if it can reactivate the injured enzyme or speed up the blocked enzyme line in another manner, such as activation of an alternate enzyme pathway. Thus, antagonism results when the stimulatory effect of one cation overcomes the toxic effect of another.

2. Acclimation and Sodium Inhibition

Acclimation of an organism to the inhibitive effects of any material is a generally observed phenomenon for cation inhibition. Thus it is not surprising that this phenomenon was observed in this study. Acclimation was achieved in this study by slowly raising the cation concentration in the environment in the daily feed studies. The concentration of a cation which could be tolerated was raised considerably by acclimation. In addition it was found that antagonism was evident even when a considerable degree of acclimation was achieved.

The results of daily feed Run 5 indicated that sodium exerted two types of inhibitive effects. In addition to the main inhibitive effect observed in this study, i.e., that of reduction in the rate of utilization of the substrate, it was found that sodium served as a synthesis block. The sodium produced synthesis block became evident at lower concentrations than the substrate utilization block. Other differences between these two inhibitive phenomena are:

(1) The substrate utilization block could be acclimated to while the synthesis block could not. (2) Potassium could completely antagonize the synthesis block, but could only partially alleviate the substrate utilization block.

The above findings give an indication of the complex nature of cation effects.

3. Application to Anaerobic System Design and Operation

The main purpose of this investigation was the delineation of the concentration ranges of the five cations studied in which acetate fermenting organisms could adequately function. Two points in these ranges are most significant: the optimum level for stimulation and the concentration at which toxicity will occur.

Optimum Ionic Environment

The discussion in the previous section indicated that stimulation was intimately associated with antagonism. Thus, peak stimulation should be obtained at the same concentrations as that producing peak antagonism. Thus, for the monovalent cations, the optimum concentration is 0.01$\underline{M}$, while for the divalent cations it is 0.005$\underline{M}$. As combinations produced results superior to antagonism by single cations, the optimum ionic environment in a digester would result when the digesting liquor contains 0.01$\underline{M}$ each of sodium, potassium and ammonium and 0.005$\underline{M}$ each of magnesium and calcium.

Toxicity

Deviation of the concentration of any of these cations from its optimum will produce a reduction in the efficiency of operation. If the cation concentration is too low, significant decreases in efficiency will result from slight changes in the cation concentration. This situation is easily rectified, however, by addition of sufficient salt to bring the cation concentration to its proper level.

Deviations on the high side will, on the other hand, produce only slight decreases in efficiency over a rather wide concentration range. Eventually, a concentration will be reached, however, where a rapid fall in efficiency will result with increase in cation concentration. This situation is critical because it is normally not economical to remove the excess cation.

This point where the cation concentration is so high that inhibition due to excess cation occurs can be considered the upper limit of cation concentration which a waste can contain and still be treatable anaerobically.

This concentration is a function of several factors: the concentration and number of antagonists present in the medium, the method of cation introduction, the organic loading and the biological solids retention time. In this study, evaluation was made for a slug addition of the cations in the absence and presence of antagonists. These results are presented in Table 14.

TABLE 14

UPPER LIMIT OF CATION CONCENTRATION FOR SLUG ADDITION OF SALTS

	Molar Concentration	
Cation	Single Cation System	Antagonists Present
Na	0.2	0.3 - 0.35
NH_4	0.1	0.25
K	0.09	0.15 - 0.2
Ca	0.07	0.125 - 0.15
Mg	0.05	0.125

It should be expected that as the organic loading is decreased and the biological solids retention time increased, the upper limit of the cation concentration for treatability will increase, because the organisms will be under less of a stress condition. If the toxic cation is introduced continuously rather than on a slug basis, acclimation should also increase the upper limit of the allowable cation concentration.

It is clear than an effort must be made to adjust the cation concentrations in an anaerobic waste treatment process as much as possible toward the optimum concentrations for each cation. This may not be possible for all cations, but the concentration of as many as possible should be adjusted to the optimum. Special care should be taken to avoid the presence of synergistic pairs in the medium. This will necessitate care in the selection of a buffering material if sufficient buffer is not present naturally. Perhaps some of the controversy over the effectiveness of various buffering materials resulted because addition of a particular material resulted in a synergistic combination in one case, and an antagonistic combination in another.

Sewage Sludge Digestion

It is commonly held that sewage sludge contains adequate quantities of all substances needed for optimum growth of methane bacteria. The results of these studies indicate that in areas where the sodium and potassium concentrations in the water supply are low, this may not be true. The current trend toward prethickening sludge tends to increase the concentrations of ammonium, calcium and magnesium, but does not increase the concentrations of sodium and potassium. Thus, sufficient quantities of sodium may not be present to antagonize the inhibition which may result from the increased ammonium concentration.

Even if ammonium toxicity does not result, the concentrations of sodium and potassium necessary to fulfill their nutritive function at the higher organic loading of high rate digestion may not be present. To prevent such a situation, the concentration of sodium and potassium should be increased if necessary. This is perhaps best done by the addition of the chloride salts of these cations.

Cation Analyses of Wastes

As synergistic and antagonistic effects are evidenced at very low concentrations, it is clear that cation analyses of wastes for which anaerobic treatment is considered would be advisable. Only with knowledge of all of the cations present and their concentrations can an evaluation of possible cation effects be made, and corrective measures be taken.

F. Conclusions

1. Cation effects in anaerobic treatment are a function of the types and concentration of all cations present in the environment.
2. An optimum ionic concentration for maximum efficiency of anaerobic treatment was found to be as follows: 0.01M for the monovalent ions, sodium, potassium and ammonium; and 0.005M for the divalent ions, calcium and magnesium.

3. Ionic concentrations either lower or higher than the optimum result in less than maximum efficiency.
4. On a molar basis, the order of increasing inhibition of the cations studied when in excess of the optimum concentration was sodium, ammonium, potassium, calcium, and magnesium.
5. The inhibition caused by an excessive concentration of any one of the ions studied could be antagonized (minimized) by the addition of the optimum concentration of at least one of the other four cations as shown in Table 12.
6. Maximum antagonism of an inhibitory cation was obtained by the addition of the optimum concentration of several rather than only one of the other cations.
7. The addition of certain of the cations to an inhibitory concentration of another cation sometimes had a synergistic effect, that is, increased the inhibition (see Table 13).
8. In many cases the synergistic cation was deterimental at concentrations well below its own inhibitory level.
9. The complicating influence of multiple cation effects is probably the reason for the varying results from anaerobic digestion studies on cation effects reported in the past.
10. Maximum efficiency from anaerobic treatment can be obtained by maintaining the major cations as close to their optimum values as possible.
11. Antagonistic relationships determined from slug feed studies are applicable to daily feed waste treatment operation.
12. A degree of acclimation to an inhibitory cation can be achieved by slowly raising the cation concentration in daily feed systems.
13. Addition of an antagonistic cation is beneficial even after maximum acclimation has taken place in a waste treatment system.

III. SULFIDE EFFECTS

One of the important inorganic materials that has been found toxic or inhibitory to methane fermentation is the sulfide ion. Sulfide ions can be produced during methane fermentation from anaerobic sulfate reduction or from protein degradation. Hydrogen sulfide is quite toxic to most organisms and thus can have a detrimental effect on methane fermentation. Since many industrial wastes have high concentration of sulfates, sulfide toxicity can be expected to be a significant phenomenon which may limit the use of methane fermentation for treatment of these particular wastes. The effects of high sulfide concentrations on anaerobic treatment were investigated in this study.

A. Previous Studies

Reports on the role of sulfides in anaerobic treatment have appeared in the literature at various times. Rudolfs and Zeller (29) reported the effects of several sulfate salts on the digestion of seeded fresh solids. Sulfate reduction to sulfide was indicated. A decrease in gas production and in volatile matter reduction occurred, the extent varying from salt to salt. In general, gas production was retarded more severely than volatile matter reduction. In connection with the treatment of white water wastes, Rudolfs and Amberg (30) investigated the effect of soluble sulfide on the digestion of seeded white water and sewage sludge. In the case of sewage sludge, 200 mg/l of sulfide decreased gas production by 70 percent, and volatile matter reduction by 50 percent. From a slug study on acetate fermentation, it was concluded that methane fermentation was a linear function of soluble sulfide concentration, and that no methane was formed at sulfide concentrations above 165 mg/l of sulfide. Soluble sulfide was felt to have little effect on volatile acid production. Aulenbach and Heukelekian (31) studied the effects of sulfide on digestion of seeded fresh solids at 20°C. At 100 mg/l Na_2S as S gas production was retarded by 50 percent compared to a control. The solubility and concentration of the sulfide salts used were considered important in determining the effect of sulfide on digestion. The methane organisms were felt to be most severely affected.

All of the above results were based on single slug-feed studies conducted at various temperatures without pH control. Therefore, their relevance to modern digester operation with daily feed and mixing is limited.

B. Theoretical Considerations

An evaluation of the possible effect of waste sulfides on anaerobic treatment must consider (1) the total quantity of sulfides either entering the digester or produced during treatment, and (2) how the sulfides are distributed within the digester. These two factors are discussed in the following.

Quantity of Sulfides

Sulfides in anaerobic treatment can result from (1) introduction of the sulfides with the raw waste, and/or (2) biological production in the digester from reduction of sulfates and other sulfur-containing inorganic compounds, as well as from anaerobic protein degradation. One of the major sources of sulfides in digesters results from sulfate reduction. Biological reduction of sulfates has been considered extensively by microbiologists (32), and also in sanitary engineering in connection with the corrosion of concrete sewers (33). The digester environment fulfills the conditions necessary for sulfate reduction, namely, low ORP, organic matter, proper temperature, and sulfates, as described by Heukelekian (34). The use of sulfate-reducing organisms to achieve waste stabilization in lieu of methane fermentation has been studied by Pipes (35). The principal organism involved in sulfate reduction is believed to be Desulfovibrio desulfuricans. This organism is a strict anaerobe. It derives energy for synthesis and maintenance from the metabolism of organic matter and uses sulfate as its terminal electron acceptor. The reduction of sulfate in this process may be expressed by the following equation:

$$8H^{+} + 8e^{-} + SO_4^{=} \xrightarrow[\text{bacteria}]{\text{sulfate-reducing}} S^{=} + 4H_2O \qquad (1)$$

The reduction of one mole of sulfur (32 grams) by this process corresponds to the oxidation of 8 equivalents of organic matter, or approximately 64 grams on a COD or oxygen equivalent basis.

A proper evaluation of the potential effect of sulfides on anaerobic waste treatment must consider the total potential quantity of sulfide in the digester. As indicated above, this includes the sulfides entering the waste, the sulfides resulting from inorganic sulfur reduction, and the sulfides resulting from protein decomposition.

Distribution of Sulfides

Sulfides in the digester may be present in a soluble or insoluble form, depending upon the cations with which they are associated. Several of the common metal sulfides such as iron, copper, zinc and nickel are insoluble and thus, heavy metal cations quantitatively precipitate and remove the sulfides from solution. The remaining soluble sulfides may assume several forms in aqueous solution, depending upon the pH. The sulfide reacts with the hydrogen ions in the solution to establish an equilibrium governed by the dissociation constants of hydrogen sulfide, a weak acid. Thus, it is possible to have H_2S, HS^-, and $S^=$ in solution. At the near neutral pH required for anaerobic treatment, only the first dissociation of hydrogen sulfide is of importance as follows:

$$H_2S \rightleftarrows H^+ + HS^- \tag{2}$$

The mass action equation for this equilibrium is:

$$\frac{[H^+]\,[HS^-]}{[H_2S]} = K_1 = 9.1 \times 10^{-8} \text{ @ } 18^\circ C^{(36)} \tag{3}$$
$$= 14.9 \times 10^{-8} \text{ @ } 35^\circ C$$

Because of the solubility relationship of hydrogen sulfide gas, a certain portion of that formed will escape with the digester gas produced. The concentration of hydrogen sulfide in the digester supernatant liquor in equilibrium with that in the gas above, is governed by Henry's Law, as follows at 35°C. (36):

$$\text{mg/l } H_2S \text{ aqueous} = 1.831 \; (\text{mg/l } H_2S_{gas})$$

A consideration of the relationship between pH and the different forms of soluble sulfide, as well as the solubility of hydrogen sulfide gas itself, allows prediction of the distribution of sulfide between the gas and aqueous phases as follows, where the total concentration of soluble sulfide is expressed as [T.S.S. aq]:

$$[\mathrm{T.S.S.\ aq.}] = [\mathrm{HS}^-] + [\mathrm{H_2S\ aq.}] \qquad (5)$$

Substituting for $[\mathrm{HS}^-]$ from Equation 3 yields:

$$[\mathrm{T.S.S.\ aq.}] = \frac{K_1\ [\mathrm{H_2S\ aq.}]}{[\mathrm{H}^+]} + [\mathrm{H_2S\ aq.}] \qquad (6)$$

Substituting for $[\mathrm{H_2S\ aq.}]$ from Equation 4 then yields:

$$\frac{[\mathrm{T.S.S.\ aq.}]}{[\mathrm{H_2S\ gas}]} = 1.831\ \left(1 + \frac{K_1}{[\mathrm{H}^+]}\right)\ @\ 35^\circ\mathrm{C} \qquad (7)$$

From the above equation, it is possible to calculate the equilibrium ratio for the concentration of soluble sulfides in the digester supernatant to the concentration of hydrogen sulfide in the gas, knowing only the digester pH. These equations could be modified to include the effect of ionic strength on the activities of the various ions, however, this modification did not appear to be justified because of other more significant variables which would prevent a complete equilibrium between the gas and liquid phases from taking place in a digester.

C. Experimental Procedure

The experimental portion of this study was conducted (1) to determine the effect of various concentrations of soluble sulfide on anaerobic treatment, (2) to determine the effect of insoluble sulfide on anaerobic treatment, and (3) to attempt to experimentally verify the soluble sulfide relationship developed above. It was desired to have the experimental conditions simulate modern full-scale digester operation as closely as possible. To achieve this end, a series of 1.5 liter laboratory digesters connected as shown in Figure 2 of the previous study, were operated on a 20-day detention time at 35°C. The digesters were mounted on a shaking platform to obtain continual mixing of the digester contents. The gas produced was collected for measurement over an acidified salt solution and expelled daily. The digesters were fed with Menlo Park, California, primary settled raw sewage sludge diluted approximately 1:1 with tap water. A volume equal to the sludge addition volume of 75 ml was withdrawn daily for analysis. The average characteristics of the raw sludge feed during this study is shown in Table 15.

TABLE 15

RAW SLUDGE FEED CHARACTERISTICS

Total Solids	1.73%
Volatile Solids	80% of Total
COD	24,000 mg/l
Loading Rate	0.05 lbs. v.s./day FT.^{3}

To establish equilibrium operation conditions, the digesters were operated for 60 days prior to the beginning of sulfide addition. Daily introduction of sulfides was accomplished by combining 5 ml of sulfate solution with the daily raw sludge feed. These solutions were prepared from Na_2SO_4 for soluble sulfide studies, and from $FeSO_4$, acidified with HCl to prevent precipitation of air-oxidized iron, for insoluble sulfide studies. The use of sulfide precursors rather than sulfide-containing compounds simulated the expected major source of sulfides in the field, and also avoided the many problems inherent in the use of a solution of soluble sulfides.

Four digesters were used in this study and the initial sulfur concentrations in the daily feed were: (1) 200 mg/l Na_2SO_4 as S; (2) 400 mg/l Na_2SO_4 as S; (3) 400 mg/l $FeSO_4$ as S; and (4) 0 mg/l S (control). After 85 days of operation, the sulfide addition rate in the 400 mg/l S unit was increased to 800 mg/l Na_2SO_4 as S for 62 days, and then to 1200 mg/l Na_2SO_4 as S. The feed rate in the initial 200 mg/l S unit was increased to 1600 mg/l Na_2SO_4 as S after 57 days of operation. The $FeSO_4$ as S unit and the control were unchanged throughout the study. The aliquots of digester contents withdrawn daily were frequently subjected to routine analysis for process control. Analyses performed were pH, alkalinity, total volatile acids by extraction technique (37), total and volatile solids, and digester gas analysis by gas chromatography. Total and soluble sulfides were determined according to the titrametric method for sewage (38), using a 25-ml sample. For soluble sulfides, the samples were placed immediately after collection into centrifuge tubes containing 0.1 ml each of 6 $\underline{N}$ $AlCl_3$, and 6 $\underline{N}$ NaOH. The completely filled tubes were stoppered and centrifuged for 5 minutes at low speeds (4 to 5 x 10^3rpm). A 25-ml volume of the centrifuged supernatant was subjected to the recovery technique employed for total sulfides. The sulfide recovery efficiency, exclusive of the centrifugation step, was found to be in the 92 to 100% range as determined from a series of known Na_2S solutions having sulfide concentrations as S of 0 to 1100 mg/l. Na_2SO_4 as S in the digester waste gas was determined by expelling the collected gas through a zinc acetate solution, and then proceeding with the iodine-thiosulfate back titration. All sulfide values presented are as directly measured.

D. Experimental Results

The average daily gas productions for the control unit and the units receiving 200 and 400 mg/l Na_2SO_4 as S, respectively, are shown in Figure 42. For the 54-day period shown, the average daily gas production expressed as a percent of the control gas production was 96.2% for the 200 mg/l S unit, and 94.2% for the 400 mg/l S unit. Volatile acid concentrations for these units were comparable to those in the control, the maximum being 72 mg/l as acetic acid. The expectation that the added sulfate would be reduced rapidly by biological action was borne out by the sulfide analyses performed on the digester supernatant and gas samples. Concentrations of total and soluble sulfides, as well as sulfides in the gas (all as sulfur) for the 400 mg/l S unit and the control are shown in Figure 43.

The constant difference between the total and soluble sulfide concentrations attained after 21 days is essentially equal to the constant difference between soluble and total sulfides in the control. This indicates that the total sulfide formed from sulfate reduction existed in the soluble form. A similar relationship between the sulfide formed was obtained in the 200 mg/l S unit. Equilibrium soluble sulfide concentrations were 32 mg/l S, and 78 mg/l S in the 200- and 400 mg/l units, respectively.

Figure 44 shows the average daily gas production and sulfide concentrations in the units receiving 800, 1200, and 1600 mg/l S., respectively. Average daily gas productions for the 800 mg/l S unit was 88.9% of the control gas production over the study period. Measured soluble sulfide reached 200 mg/l S. At no time in the 62 days of operation did the volatile acid concentration in this unit rise above 58 mg/l as acetic acid.

When the sulfur concentration of the 800 mg/l S digester feed was increased to 1200 mg/l, toxic conditions resulted. Gas production ceased within 40 days. The soluble sulfide concentration rose to 384 mg/l S by the 34th day. Initially, the volatile acid concentration increased slowly, reaching 380 mg/l on the 21st day, and 1050 mg/l by the 33rd day. Then the rate of accumulation increased, resulting in a final concentration of 1910 mg/l as acetic acid on Day 40. As shown in Figure 44, toxic conditions resulted when the sulfide concentration in the 200 mg/l sulfide unit was increased to 1600 mg/l. Measured soluble sulfide concentrations reached 495 mg/l S.

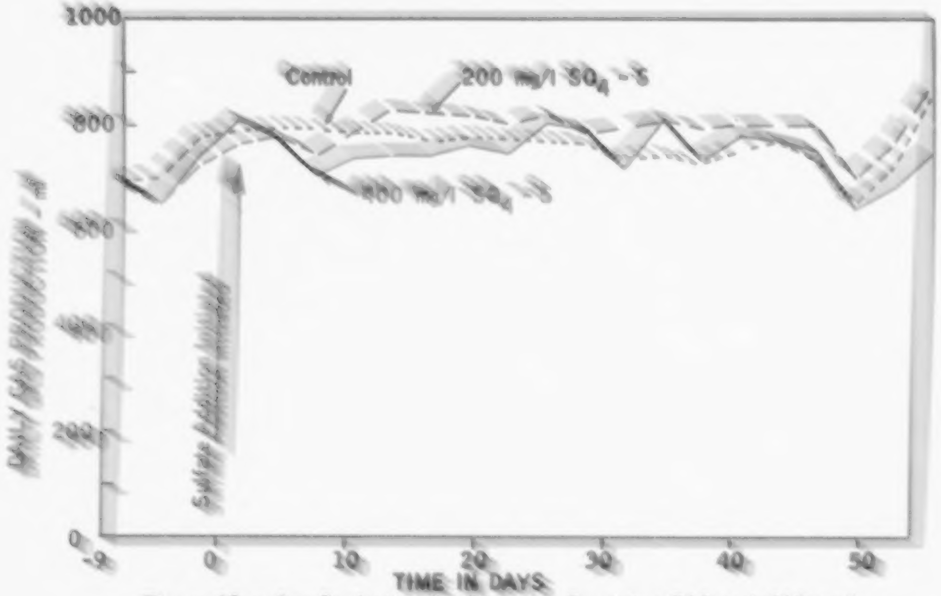


Figure 42 - Gas Production for Digesters Receiving 200 and 400 mg/l Sulfate Sulfur and Control

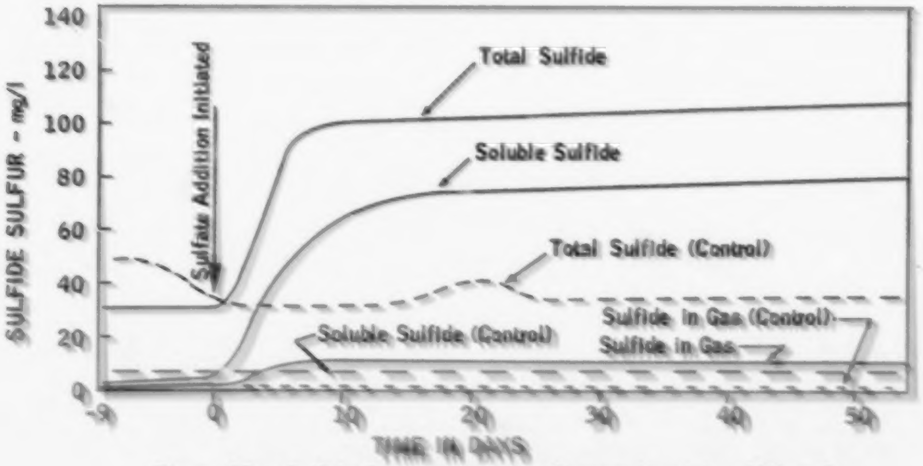


Figure 43 - Sulfide Concentrations for Digester Receiving 400 mg/l Sulfate Sulfur and Control

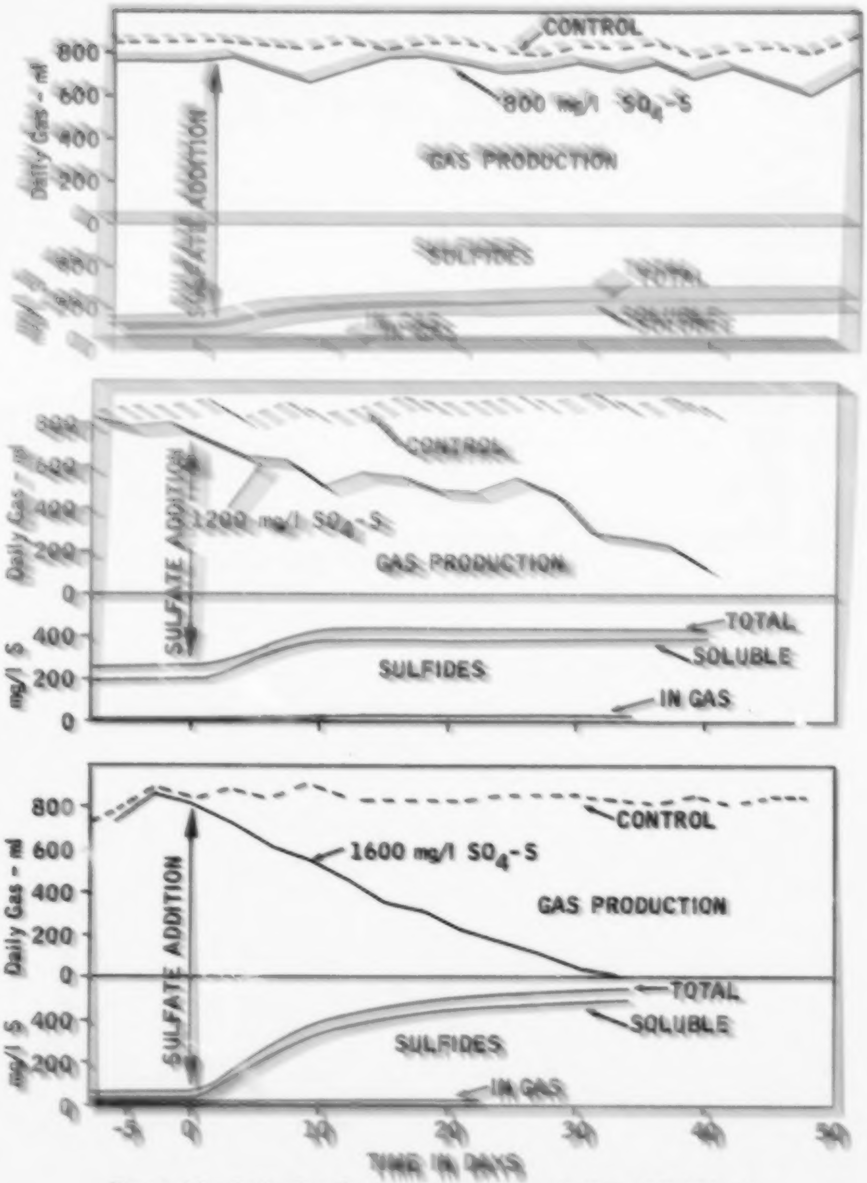


Figure 44 - Daily Gas Production and Concentration of Sulfides for Digesters Receiving 800, 1200, and 1600 mg/l Sulfate Sulfur

A plot of volatile acid concentrations and gas production for this unit is shown in Figure 45. It can be seen that gas production declined to less than 50 percent of the control before a significant increase in volatile acids resulted. Thus, it appears sulfides initially affected the acid producing organisms as much or more than the methane formers. This also indicates that an increase in volatile acids would not be a reliable indicator for the onset of sulfide toxicity.

The pH and alkalinity of the digester mixed liquor at the termination of each phase of the study, along with predicted increases in alkalinity due to reduction of sulfate (strong acid) to sulfides (weak acids) are shown in Table 16. The close agreement between predicted and measured alkalinities is a further indication that total reduction of sulfates to sulfides was accomplished throughout these studies.

TABLE 16

pH, INCREASE IN, AND TOTAL ALKALINITY OF DIGESTERS

Feed SO_4 - S mg/l	pH	Calculated Alkalinity Increase	Calculated Alkalinity Total	Measured Alkalinity
(0) Control	7.0	0	--	1650
200	7.1	620	2270	2100
400	7.1	1250	2900	2900
800	7.3	2500	4150	4400
1200	6.9	3750	5400	5100
1600	7.2	3333	4983	4800

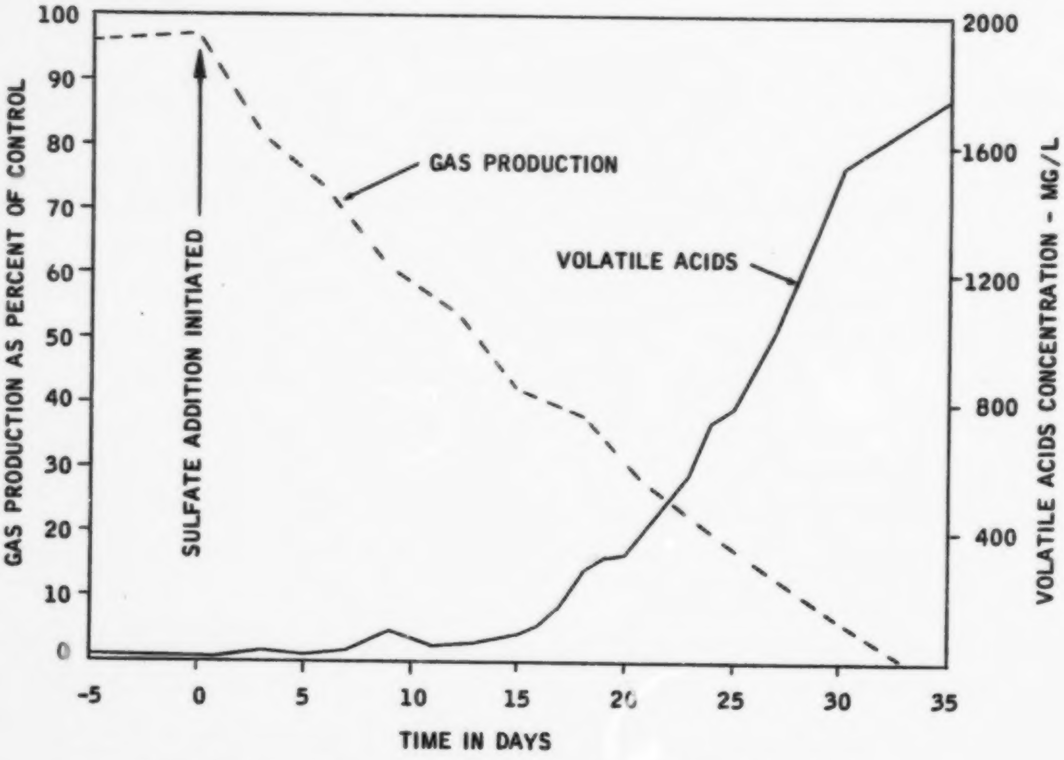


Figure 45 - Gas Production and Volatile Acids Versus Time for Digester Receiving 1600mg/l Sulfate Sulfur

Average daily gas production and sulfide concentrations for the digester fed 400 mg/l $FeSO_4$ as S are shown in Figures 46 and 47. The average daily gas production over the study period was 91.0% of the control for the same period. Volatile acid concentrations were comparable to those in the control with the maximum being 50 mg/l as acetic acid. Here, the unit with iron to precipitate the sulfides when formed operated very well, even with total sulfide concentration as high as 400 mg/l. This indicates that only the soluble sulfides are inhibitory to anaerobic fermentation, and not the insoluble sulfides.

E. Discussion

Sulfide Toxicity

Soluble sulfides can produce toxic or inhibitory effects in anaerobic treatment as shown by the experimental results of this study. This toxicity is initially evidenced by a decrease in gas production and finally in severely retarded digestion, by an increase in volatile acids concentration. The decrease in gas production was not significant (less than 12%) in the digesters having equilibrium soluble sulfide concentrations up to 200 mg/l S. This latter concentration occurred in the digester fed 800 mg/l Na_2SO_4 as S. In the two laboratory units containing greater than 200 mg/l soluble sulfide, the toxic effects resulted in total cessation of gas production and a volatile acid accumulation.

The results of this study indicate that the relationship between gas production as a percent of the control and soluble sulfide concentration is not linear. The gas production decreased gradually with increasing sulfide concentration up to a "threshold" value, beyond which severe toxicity and total cessation of gas production resulted. From this study the "toxic threshold" for soluble sulfide appears to lie between 200 and 400 mg/l S. This type of toxic effect agrees with the rather broad zone of slightly sub-optimal operation noted by Kugelmann and McCarty (2) for cation toxicity. It is at variance with the linear relationship derived from slug studies by Rudolfs and Amberg (4). Such a discrepancy points out the difficulty, at least with sulfides, in extrapolating results of uncontrolled slug studies to continuous operation. The daily addition of sulfides or sulfide precursors to digesters under conditions more closely approximating full-scale operations presents a much different picture of the tolerance of anaerobic treatment to soluble sulfides.

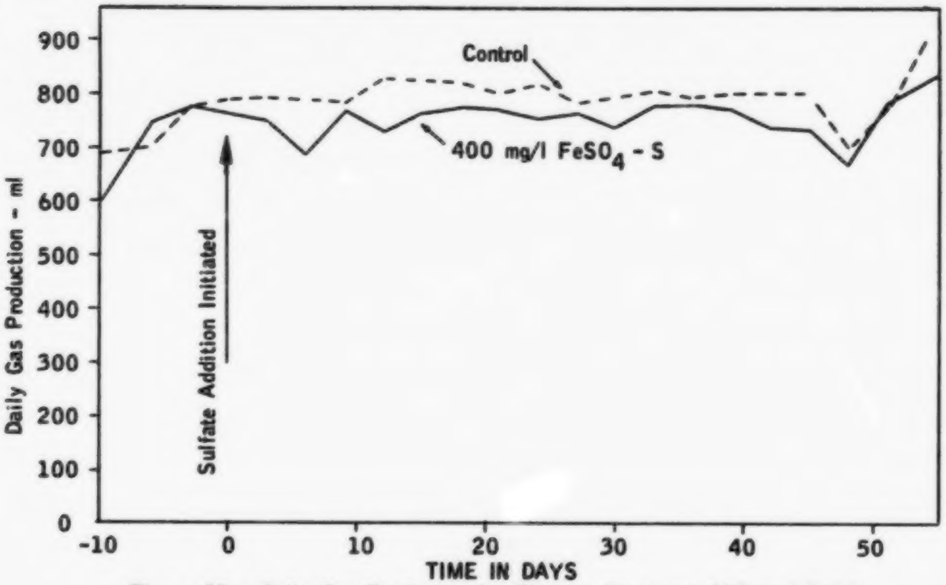


Figure 46 - Daily Gas Production for Digester Receiving 400 mg/l $FeSO_4$ Sulfur

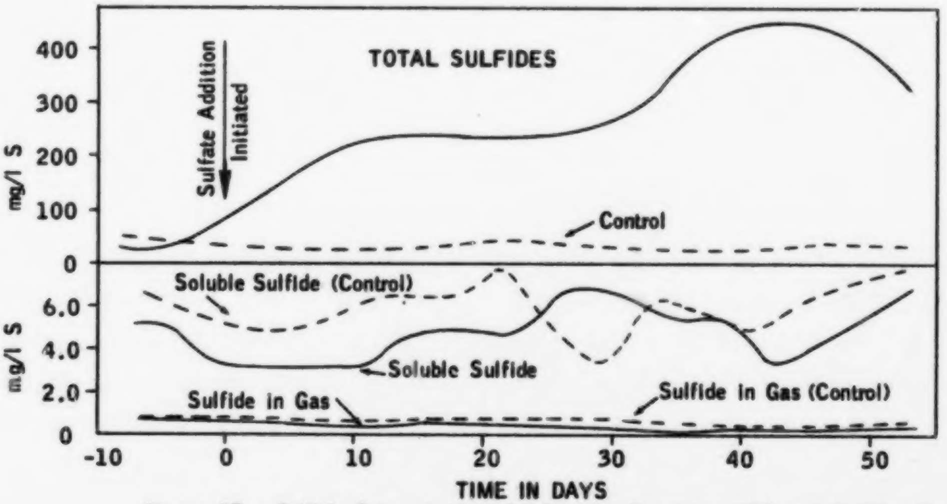


Figure 47 - Sulfide Concentrations for Digester Receiving 400 mg/l $FeSO_4$ -S and Control Digester

Also, as indicated by others, insoluble sulfides appear to exert relatively minor effects on anaerobic treatment as evidenced by the results obtained from the digester receiving 400 mg/l $FeSO_4$ as S. The iron sulfides formed would be insoluble as evidenced by the solubility product of the most probable form present, ferrous sulfide:

$$K_{sp} = 3.7 \times 10^{-19} \text{ at } 18^{\circ}C.$$

That this was the case is evident from the results of the sulfide analyses. These analyses showed that although the soluble sulfide concentration was lower than in the control, the total sulfide concentration reached a high value of 445 mg/l S. Even with this high sulfide concentration, no toxicity resulted.

A comparison of the performance of this digester to the digester fed 1200 mg/l S illustrates the role of solubility of the sulfides in determining the effect of sulfides on anaerobic treatment. The total sulfide concentrations in these two digesters were similar. However, gas production had completely terminated in the digester where the sulfides were almost all soluble, while the digester which contained this quantity of sulfides as the insoluble ferrous sulfide was almost unaffected. Thus, it appears that insoluble iron sulfides in concentrations up to at least 400 mg/l do not seriously affect anaerobic treatment, and that only soluble sulfide concentrations need to be considered in assessing the effects of sulfides on digester operation.

Sulfide Solubility

The quantity of soluble sulfides that will result in solution from waste treatment is not only related to the total quantity entering and produced within the digester, but is also related to the quantity which will be lost through precipitation by heavy metals and to that which escapes with the gas. The quantity of soluble sulfide which escapes with the gas is quite considerable, as evidenced by the results of this study. For example, the soluble sulfide concentration in the unit fed 800 mg/l Na_2SO_4 as S was only 1/4th of the feed concentration, or approximately 200 mg/l S. This large reduction in soluble sulfides is almost entirely due to the sulfide lost by the daily expulsion of gas. The quantity of sulfides lost in the gas is related to the relative quantity of gas produced each day, as well as the relationship between aqueous soluble sulfide and gaseous hydrogen sulfide as given in Equation 6.

The relationship between the quantity of soluble sulfides entering or formed within the digester and the quantity which is lost in the gas is as follows:

$$V_1S_s = V_gS_g + V_1S_e \tag{8}$$

where,
V_1 = Volume of waste entering or leaving digester per day,
V_g = Volume of gas produced per day,
S_s = Concentration of soluble sulfides entering or formed within the digester,
S_g = Sulfide concentration in gas produced,
S_e = Soluble sulfide concentration in digester effluent.

The ratio of soluble sulfides in the digester effluent to that in the gas at equilibrium is rated to Equation 6 as follows for 35°C:

$$\frac{S_e}{S_g} = \frac{[T.S.S._{aq.}]}{[H_2S_{gas}]} = 1.831 \left(1 + \frac{K_1}{H^+}\right) = A \tag{9}$$

Here, A is a constant for any given pH and temperature. Combining Equations 8 and 9 gives:

$$V_1S_s = V_gS_e/A + V_1S_e \tag{10}$$

or,

$$\frac{S_e}{S_s} = \frac{1}{1 + V_g/AV_1} \tag{11}$$

The relationships between pH and the ratio of total soluble sulfide concentration in the waste during treatment and hydrogen sulfide concentration in the gas calculated from Equation 9, for three different temperatures, are shown in Figure 48. Temperature can be seen to have only a small influence. Experimental ratios determined from measured sulfide values are also shown in this figure. The experimental values lie somewhat above the theoretical curve in all cases. Closer agreement would have been obtained if activity rather than molar concentration were used in Equation 9.. In addition, some removal of hydrogen sulfide in the gas due to absorption into the confining fluid in the gas collection tubes tended to give higher results than expected. It would also be expected that without continuous feed and complete mixing between the gases and supernatant liquor phase, complete equilibrium would not be obtained, causing a discrepancy between the theoretical and measured values. In spite of these differences, however, the experimental data adequately substantiates the developed relationship of Equation 9.

Equation 11 is shown graphically in Figure 49. Here, the influence of gas production and sulfide solubility (A) on the fraction of soluble sulfides remaining in solution can be seen. From Figure 48, it can also be seen that values of A varying from 4 to 8 could be expected for normal digester operation near a neutral pH. With these values, Figures 49 indicate that the fraction of soluble sulfide remaining in solution is inversely proportional to the volume of gas produced per unit volume of waste. Since more concentrated wastes will tend to produce more gas per unit volume, the fraction of sulfides remaining in solution for these wastes will tend to be lower. Thus, higher sulfide production can be tolerated in more concentrated wastes as larger portions will be removed from solution by the greater quantities of gas produced.

For example, a 3 percent waste would have a ratio of gas production per unit volume of waste of about 20, and the fraction of soluble sulfides remaining in solution would be about 25%. For comparison, a 1 percent waste would have a ratio of about 7, and the fraction of soluble sulfides remaining would be about 45%. One other important point is that once the gas production began to decrease due to sulfide inhibition, less sulfides would be driven from solution, the sulfide concentration would then rise higher, causing more inhibition. This cyclic toxic effect was observed in this study and rapidly results in total cessation of gas production.

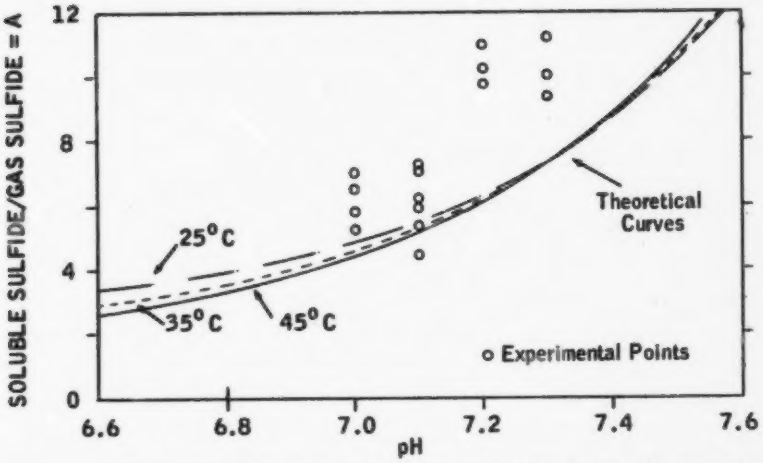


Figure 48 - Effect of pH on Ratio of Soluble Sulfide Concentration to Sulfide Concentration in Gas

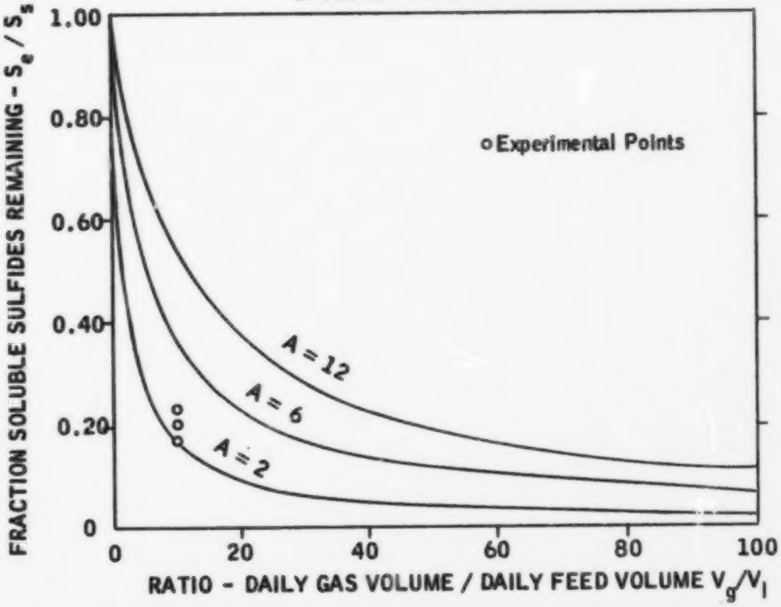


Figure 49 - Effect of Operational Variables on Fraction of Sulfides Remaining in Digester Effluent

Experimental points representing average values from this study are shown in Figure 49. These points are for operation at pH of 7.1 and 7.3, and the calculated A values varied from about 4.5 to 7. The points can be seen to be a little low, due mainly to the fact that a fair portion of the sulfides were removed from the gas by absorption into the gas retaining fluid. The theoretical values represented by the drawn curves would be more conservative and are what would be expected in field operation.

Precipitation of Toxic Sulfides

The previous studies have shown that relatively high sulfur concentrations can be tolerated in a daily fed digester receiving a concentrated waste, since a good portion of the sulfides are removed along with the gas produced. One significant problem from removal of sulfides with the gas, is that the gas would be quite corrosive, and gas-handling facilities would have to be well protected. Another way for preventing a toxic sulfide concentration would be to precipitate the sulfides with iron either externally by gas recirculation or internally by adding iron compounds with the wastes. This is suggested from the the results of the digester-fed $FeSO_4$. Here, concentrations of sulfide up to 400 mg/l S could be tolerated with only minor effects on the operation of the treatment process. Such an approach might be beneficial for more dilute wastes. An amount of iron salts such as ferric chloride equivalent to the waste sulfide molar concentration could be added to the digester. It might also be necessary to add sufficient base to the system to compensate for the decrease in alkalinity from ferric chloride additions.

The continuous addition of iron salts for sulfide control should give few operational problems. Current studies have demonstrated that addition of an excess of iron, sufficient to precipitate up to 800 mg/l of sulfide, could be added to a digester with relatively minor effect on gas production or volatile acid production. This iron addition was over and above that required to precipitate the sulfides present and thus indicates that iron addition in excess of sulfide concentration in the waste should have no serious effect on anaerobic treatment. Thus, excessive iron concentrations are not harmful. The use of iron to precipitate sulfides could therefore be used on a continuous basis to reduce sulfide toxicity, or else could be used on a slug basis to overcome the inhibitory effect of a sudden increase in sulfide concentration.

F. Summary and Conclusions

The effects of soluble and insoluble sulfides on anaerobic treatment have been investigated by the operation of a series of laboratory digesters receiving daily sulfide additions. A theoretical discussion of the chemical relationships covering the species of soluble sulfide and concentrations of soluble sulfide in the aqueous and in the gas phase have been presented. Experimental results have been discussed with respect to toxic concentrations of soluble sulfides and also with respect to agreement with the developed relationships. Possible methods for controlling or eliminating the sulfide toxicity have been discussed. The following conclusions may be drawn from this study:

(1) Equilibrium concentrations of soluble sulfide up to at least 200 mg/l S exert no significant toxic effects on anaerobic treatment, with daily feed operation.

(2) Concentrations of soluble sulfide higher than 200 mg/l S somewhat inhibit gas production and such concentrations greater than 370 mg/l S produce severe toxic effects and complete cessation of gas production.

(3) Insoluble or non-acid liable complexes of sulfide have no significant effect on anaerobic treatment up to a concentration of at least 400 mg/l of sulfide.

(4) The concentration of sulfide in solution in the digester is related not only to the quantity of sulfide or sulfide precursors entering with the waste, but also to the quantity of sulfides lost with the digester gas.

(5) The quantity of sulfides lost with the daily gas is related to the solubility of hydrogen sulfide, the digester pH, and the relative daily gas production.

(6) Iron can be used to "inactivate" sulfides or reduce sulfide toxicity by precipitating sulfides from the biological environment.

IV. HEAVY METAL EFFECTS

Heavy metals have frequently been found the cause of unbalanced digestion. Also, in many other instances, heavy metals have either been blamed or suspected as the reason for digestion failure, although good evidence often has been lacking. The reported toxic concentrations for heavy metal salts have varied widely. This is perhaps due to the fact that these metals form precipitates with many compounds and so are often removed from solution and thus from the sphere of influence on the microorganisms. The methods of reporting salt concentrations has also varied widely, which has led to difficulties in comparison from one report to another.

The most prevalent heavy metals which may gain access to digesters are copper, nickel, zinc, chromium, and iron. In most cases, copper has been the heavy metal of most concern. Although concentrations of copper much less than 1 ppm are known to be toxic to microorganisms, several times this concentration have frequently been reported in digesters, with no failure. The reason for this appears to be that copper is for the most part present in an insoluble form. Recent studies by Masselli and Masselli (48) have indicated that sulfides are able to precipitate copper to such an extent that it is no longer toxic under anaerobic conditions. Since most heavy metal sulfides are extremely insoluble, and also since sulfides are frequently present in relatively high concentrations in digesters, it appears sulfide may be the major factor in preventing heavy metal toxicity in anaerobic treatment. The following study was conducted to determine the level of toxicity of various heavy metals, and to evaluate the role played by sulfides in the removal of this toxicity.

A. Previous Studies

Copper Toxicity.

Rudolfs (49) reported that copper sulfate in a concentration of 0.05 percent based on dry volatile matter (which was 2.78 percent by concentration in the sludge) almost stopped the digestion of seeded raw sludge, while a concentration of 0.037 percent had little effect. Vilen (50) found that the presence of 0.37 percent copper on a dried solids basis could be tolerated in the digesters at Kenosha, Wisconsin, provided other factors were favorable. Rugal (51) reported that with both batch-feed and continuous-feed, copper concentrations of between 150 to 250 mg/l were toxic to digestion. He (52) found in another study that gas production dropped when the copper concentration exceeded 500 mg/l. He stated that copper as copper hydroxide and as hydrated copper sulfate were both toxic to sludge digestion to about the same degree.

At an operator's forum in 1948, Wahlstrom (53) stated that the digesters at La Crosse, Wisconsin, operated with a copper concentration of 1 to 1.5 percent on a dried solids basis. Stander (54) noted definite toxic effects on sludge digestion at copper concentrations ranging from 0.41 to 1.33 percent on a dried solids basis. In a laboratory study, McDermott, Moore, Post and Ettinger (55) obtained satisfactory digestion of a mixture of primary and activated sludge with copper concentrations at times exceeding 2 percent of the digested dried solids. Above this concentration, sub-normal operation was observed. They related the toxic effects to the concentration of copper entering the treatment plant with the raw sewage. They obtained normal operation from digestion of primary sludge obtained from sewage with a copper concentration of 10 mg/l, but sub-normal operation when the sewage copper concentration was 15 mg/l. With combined primary and excess activated sludge, more of the copper entering the treatment plant precipitated and became part of the sludge and therefore less could be tolerated in the incoming sewage. Here 10 ppm of copper in the sewage caused sub-normal operation, while 5 mg/l could be tolerated.

An excellent summary of the occurrence of copper and zinc in domestic sewage and sewage sludge has been presented by Masselli and Masselli (48). They found concentrations of 150 to 500 mg/l or 0.5 to 1.7 on a dry volatile solids basis affected digestion. However, they also reported that by adding sufficient sulfides to precipitate the copper, the toxic effects were eliminated and digestion proceeded normally, even at these high concentrations. Thus, they suggested an excellent possible method for control of heavy metal toxicity.

Nickel Toxicity

Toxicity of nickel was investigated by Wischmeyer and Chapman (56). In laboratory experiments, they added nickel in the form of $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$ and $NiSO_4 \cdot 6H_2O$ in varying concentrations. They reported that with both salts, concentrations of up to 500 mg/l as nickel stimulated digestion. Concentrations in excess of this value, however, and especially higher than 1,000 mg/l, retarded digestion.

Zinc Toxicity

McDermott, Barth, Salotto, and Ettinger (47) evaluated the effect of zinc on the anaerobic treatment processes. They found sludges obtained from sewage containing 10 mg/l of zinc digested at normal rates, but when the concentration was raised to 20 mg/l, rapid failure of the digestion process occurred. The concentration of zinc in the digested sludge was 341 mg/l on a volumetric basis or 3.16 percent based on total solids. At this concentration, the zinc was not toxic. When the concentration was raised, sub-normal operation resulted.

Iron Toxicity

Setter (58) reported that ferric chloride and ferric sulfate in concentrations in excess of 100 mg/l caused a marked retardation in digester gas production, and also lowered the pH. Ferric hydroxide appeared to cause a slight stimulation and ferric oxide had no effect on the process. Iron citrate and ferrous sulfate showed a slight retardation on gas production, and ferrous acetate caused somewhat more retardation. Rudolfs (59) reported 1,000 mg/l reduced gasification materially.

Chromium Toxicity

Pagano (60) studied the effects of chromium on acetic acid digestion. He found that as much as 50 to 200 mg/l of chromic chloride as chromium could be added on a slug-feed basis without affecting gas production, but small daily increases after this slug feed hindered gasification. From continuous-feed studies, Pagano found 3 to 6 mg/l of this salt per day could be tolerated, but 10 mg/l decreased gas production after the 7th day. With hexavalent chromium, only 1.25 to 1.5 mg/l per day could be tolerated without affecting gas production. Trivalent and hexavalent chromium were both found to precipitate rapidly after addition. Vryburg (61) reported from laboratory experiments that gas production was reduced to one-half with 500 mg/l of chromium and to 1/6th with 1,000 mg/l.

Ross (62) stated trivalent chromium apparently did not hinder digestion, but hexavalent chromium was toxic. He believed that if the waste to his plant contained over 0.75 mg/l of hexavalent chromium, it would adversely affect operation. Wahlstrom (53) reported no adverse affects could be noticed even with 0.4 to 0.5 percent of chromium on a dried solids basis. Moore, McDermott, Post, Mandia and Ettinger (63) studied the effects of both daily additions and slug additions of chromium on digester operation. They found a slug addition of 300 mg/l of hexavalent chromium caused initial retardation of gas production, but the digester gradually recovered. At 500 mg/l, however, no recovery was obtained. They found that digesters containing 200 to 420 mg/l of chromium, or 2.8 to 3.46 percent on a dried solids basis were not adversely affected. The chromium in the sludge obtained from primary and secondary treatment was, for the most part, in the reduced trivalent state. High concentrations of this material apparently had no effect on digestion. However, when 50 mg/l of hexavalent chromium was fed daily directly to the digester, gas production dropped off rapidly.

Summary

Previous studies indicate that toxic concentrations of the heavy metals vary widely. For the most part the heavy metals are in an insoluble form and have relatively little effect on digestion. It also appears that high concentrations of most heavy metals can cause rapid toxicity and cessation of the digestion process.

3. Experimental Procedure

This study was conducted to determine the effect of various concentrations of several heavy metals both singly and in combination on anaerobic treatment. Also, the suspected role of sulfides in preventing heavy metal toxicity as reported by Masselli and Masselli (48) was also investigated. The heavy metals studied were the ones commonly suspected of giving problems in anaerobic treatment. These were copper, zinc, nickel and iron. The solubility products of the sulfides of these metals, as well as the solubility products of several other heavy metals are shown in Table 17. From the values shown, it can be seen copper is one of the most insoluble of the heavy metal sulfides and iron is one of the least insoluble. However, they are all quite insoluble and in the presence of sulfide it can be expected that the heavy metal concentration in solution will be practically zero.

TABLE 17

SOLUBILITY PRODUCTS OF HEAVY METAL SULFIDES (36)

Heavy Metal	Sulfide Salt	Solubility Product	Temperature °C
Copper	Cu_2S	2.0×10^{-47}	16 - 18
Copper	CuS	8.5×10^{-45}	18
Lead	PbS	3.4×10^{-28}	18
Cobalt	CoS	3.0×10^{-26}	18
Nickel	NiS	1.4×10^{-24}	18
Zinc	ZnS	1.2×10^{-23}	18
Iron	FeS	3.7×10^{-19}	18

The experimental studies were conducted using a series of 1.5-liter laboratory digesters as described under Section III on Sulfide Effects. These digesters were operated on a 20-day detention time at 35°C, and were mounted on a shaking platform to obtain continual mixing of the digester contents. Primary settled raw sewage sludge was used in this study and the average characteristics of the raw sludge feed was as shown in Table 15 for the sulfide studies. To establish equilibrium operation conditions, the digesters were operated for 60 days prior to the beginning of the heavy metal additions.

Nine digesters were used in this study. One served as a control and received only raw sludge as feed. The other eight digesters also received raw sludge as feed, but in addition a heavy metal salt was added continuously to each digester. The heavy metals studied were copper, zinc, nickel and iron. Initially, each heavy metal was fed at two different concentrations to separate digesters. The heavy metals were fed initially as the sulfate salts. It was expected that by daily feed of the sulfate salts, the sulfates would be reduced to sulfides in the digester, and precipitation of the heavy metals would then occur. This procedure was followed to simulate expected field operation where heavy metals would be added with the sludge to the digester, and subsequently be precipitated by sulfides formed from sulfate reduction within the digester. The sulfide salts of the heavy metals could have been added, but this would not as closely simulate field conditions. Addition of the sulfate salts instead allowed the heavy metals to form complexes or precipitates with other materials in the digester if this were to occur.

The concentrations of each heavy metal added to the individual digesters are shown in Table 18.

TABLE 18

HEAVY METALS ADDED TO FEED OF INDIVIDUAL DIGESTERS

Heavy Metal	Salt Added	Concentration in Feed mM	mg/1 S	mg/1 Metal
Iron	$FeSO_4 \cdot 7H_2O$	6.25	200	349
Iron	$FeSO_4 \cdot 7H_2O$	13.5	400	698
Copper	$CuSO_4 \cdot 5H_2O$	6.25	200	397
Copper	$CuSO_4 \cdot 5H_2O$	13.5	400	794
Zinc	$ZnSO_4 \cdot 7H_2O$	6.25	200	409
Zinc	$ZnSO_4 \cdot 7H_2O$	13.5	400	817
Nickel	$NiSO_4 \cdot 6H_2O$	6.25	200	367
Nickel	$NiSO_4 \cdot 6H_2O$	13.5	400	734

The heavy metal sulfates were added in concentrations of 6.25 and 13.5 mM, equivalent to 200 mg/1 and 400 mg/1 of sulfur respectively. After the digesters had been operating well, for an extended period with heavy metal sulfates, the heavy metals in some instances were then added as the chloride salt, rather than the sulfate salt. At this point no more sulfides were formed for precipitation and the effect of this change was then noted. Also, during this study operation was changed in some digesters so that a combination of heavy metals, rather than individual heavy metals were added to certain digesters to find the effect of such combinations. Individual procedures followed in the operation of each digester will be described under results.

The aliquots of digester contents withdrawn daily were frequently subjected to routine analyses for process control, as in the previous reported sulfide study. Analyses performed were pH, alkalinity, total volatile acids by extraction technique (37), total and volatile solids, and digester gas analysis by gas chromatography. Total and soluble sulfides were determined by use of the titrametric method for sewage described under the sulfide effects section.

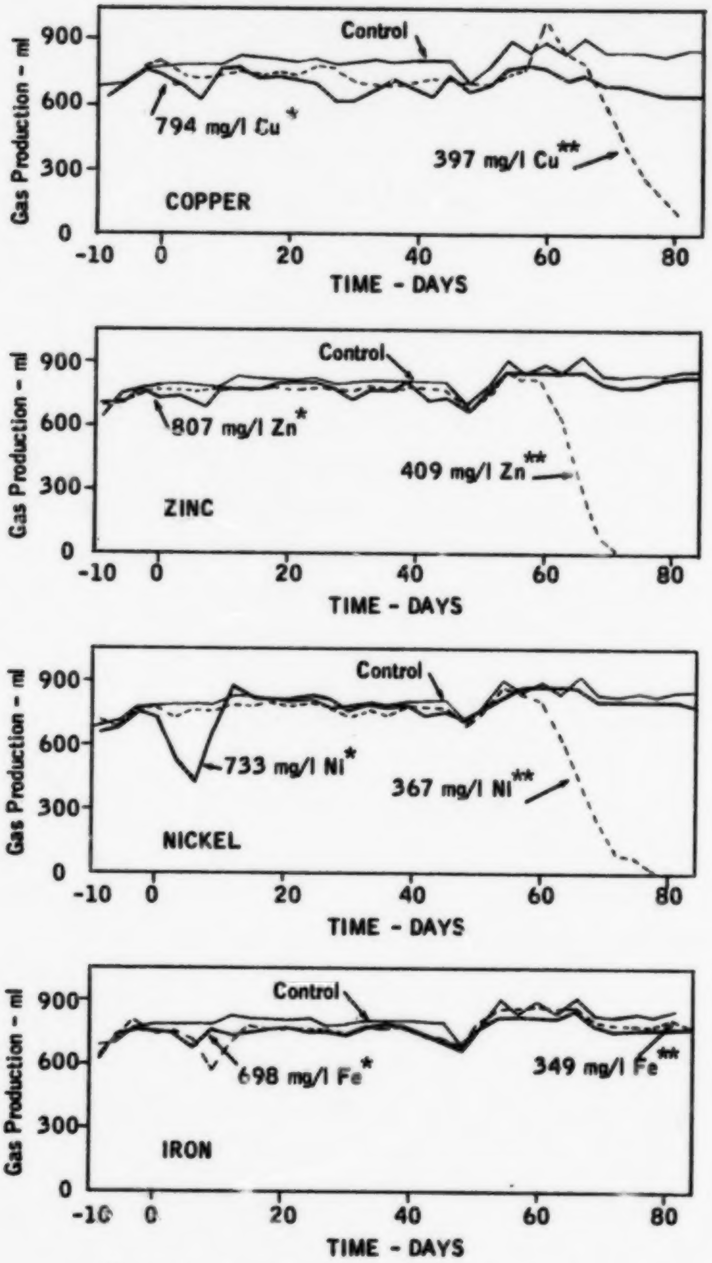
C. Experimental Results

Individual Heavy Metals

In the initial studies, concentrations of the four heavy metals, copper, zinc, nickel, and iron, were fed to eight different digesters at two different concentrations. The concentrations used as shown in Table 18 were equivalent to 200 mg/l and 400 mg/l of sulfides as sulfur, respectively. The effects of these additions on gas production are shown in Figure 50. The additions of heavy metals were continued for 57 days, corresponding to approximately 3 detention times. Throughout this period, the operation of all digesters proceeded quite satisfactorily, with gas production equaling that of the control. One unit, however, receiving 733 mg/l of nickel appeared to suffer somewhat initially when nickel was first introduced. This appeared to be due to initial incomplete reduction of sulfates. This condition was soon overcome and digestion then proceeded as normal.

In all cases, the volatile acid concentrations were low (less than 100 mg/l) and percent of methane in the digester gases was normal, (63 to 67 percent). In almost all cases, the digestion in the presence of heavy metals was the same as in the control. The sole exceptions were the units receiving copper. Here, the total gas production remained slightly below that of the control. This was especially noticeable in the unit receiving 794 mg/l of copper. Apparently the copper at this concentration caused some minor effects, although such effects were not revealed by the normal routine analyses.

As will be indicated later, the sulfates appeared to be reduced to sulfides, which then precipitated the heavy metals. Thus, the heavy metals were rendered insoluble and non-toxic. To further check to see if the sulfides were essential in the reduction of toxicity, after 57 days of operation, the sulfate additions to the units receiving 200 mg/l as equivalent sulfur, were discontinued. Here the heavy metals were then added as the chloride salts.



* Added as Sulfate Salt During Period Shown.

** Changed from Sulfate Salt to Chloride Salt on Day 57.

Figure 50 - Gas Production for Digesters Receiving Individual Heavy Metals

The units receiving the higher concentration of heavy metal sulfate salts were continued and served as controls. From Figures 50 and 51, it can be seen that as soon as the sulfate salts were replaced by chloride salts, no sulfides were formed for precipitation of the heavy metals, and the copper, zinc, and nickel digesters failed within a very short time. Gas production in all cases dropped almost to zero within 10 to 20 days. However, the unit receiving iron was unaffected by this change. Thus, it appears sulffdes are required to prevent toxicity from the heavy metals, copper, zinc, and nickel.

The change in gas production and volatile acid concentration which occurred after the sulfate additions were discontinued are shown in Figure 51. Here it can be seen that gas production was the initial parameter affected by the heavy metal toxicity. The volatile acid concentration began to rise only after the gas production was noticeably reduced. This was especially true in the units receiving nickel. Here, the gas production was only about 20 percent of the control before a noticeable increase in volatile acids occurred. Also, when the gas production reached zero, the volatile acid concentration was only 500 mg/l. With zinc and copper, the final volatile acid concentration after complete failure of digestion, was only around 1,000 mg/l. Thus, it appears the heavy metals affect the acid producing bacteria, at least as much if not more than the methane bacteria. Here, it can be seen that an increase in volatile acids is not one of the best indications of heavy metal toxicity. By the time volatile acid concentration had risen significantly, the digesters had already been seriously affected. This is another indication that all normal control parameters, rather than one alone, must be analyzed for adequate digester control.

One other analysis sometimes used to judge digester operation is gas analysis. It has often been noted that when digesters begin to fail, the percentage of CH_4 decreases significantly. The change in methane fraction which occurred after significant changes in gas production occurred in this study are shown in Table 19.

TABLE 19

EFFECT OF HEAVY METAL TOXICITY ON METHANE FRACTION OF GAS

Heavy Metal	Days After Change to Chloride Salt	Gas Production in % of Control	% Methane in Gas
Copper-397 mg/l	13	69	60
	20	24	50
Zinc -409 mg/l	5	83	67
	13	5	50
Nickel-367 mg/l	5	81	67
	13	22	67

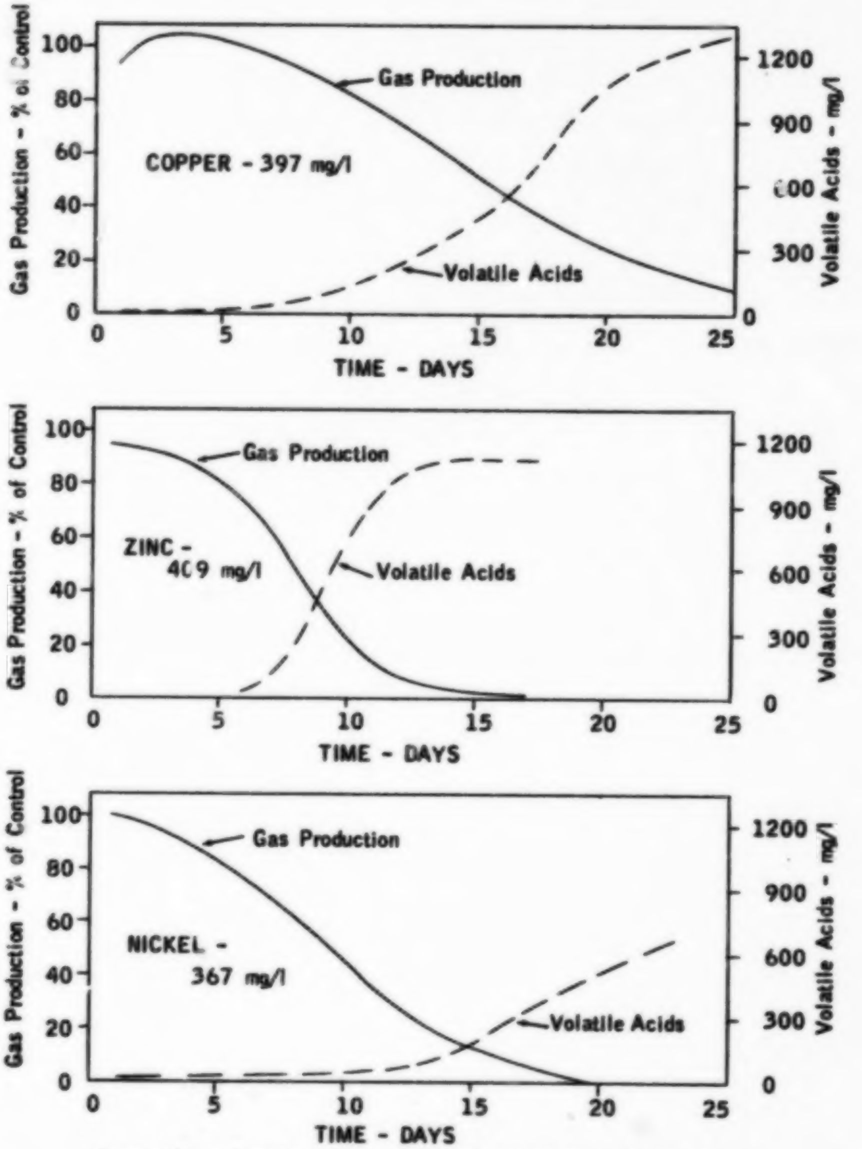


Figure 51 - Gas Production and Volatile Acid Concentration for Digesters Receiving Metals as Shown for Time Period Beginning at Termination of Sulfate Addition

The changes in methane fractions were small and were not noticeable until gas production dropped significantly. The change in methane fractions also did not occur until after a build-up in volatile acids took place. Both thus indicate the same thing, that methane bacteria are being affected to the point where they cannot handle the acids as fast as produced by the acid forming bacteria. This was apparently not the case in the nickel unit, even when the gas had been reduced to 22 percent of the control.

This initial study indicated quite well that sulfides formed from sulfate reduction are very instrumental in removing copper, nickel, and zinc heavy metal toxicity. It was shown that when sulfate addition was discontinued, the units receiving these heavy metals rapidly failed. However, units receiving twice the concentration of heavy metals continued as well as the control, as long as an equivalent amount of sulfates were added. This study also indicated that daily additions of iron were not detrimental to digestion, and this heavy metal does not require sulfide precipitation for removal of toxicity.

Combinations of Heavy Metals

In this study, combinations of heavy metal sulfate salts were introduced to the digesters. The digesters receiving the higher concentrations of heavy metal sulfate salts from the individual heavy metal study were continued in operation and switched to combinations of heavy metals for this study. The combinations were added to give a total concentration of 13.5 mM heavy metals in the feed. The concentrations of each heavy metal used are shown in Table 20. Figure 52 shows the daily gas production that resulted from these combinations.

The first graph shows the gas production from the copper-zinc combination. It can be seen that once this combination was added, gas production improved, and was then as good as in the control. Thus, with this combination, the slightly detrimental effect noted with copper alone appeared to be relieved. At the end of 31 days of satisfactory operation, the sulfates were removed from the daily feed and chloride salts of the heavy metals added. As in the previous study, this immediately resulted in failure of the digester.

The zinc-nickel combination proceeded normally for 31 days, with gas production similar to the control. After that time, the sulfates were removed and the digestion failure was rapid, with gas production reaching zero within about 12 days.

TABLE 20

CONCENTRATIONS OF HEAVY METALS IN FEED SLUDGE FOR COMBINATION STUDY

Heavy Metals	Concentration of Heavy Metals mM	Concentration of Heavy Metals mg/1
Copper-Zinc Unit:		
Copper	6.25	397
Zinc	6.25	409
Total	13.5	806
Zinc-Nickel Unit:		
Zinc	6.25	409
Nickel	6.25	367
Total	13.5	776
Iron-Zinc Unit:		
Iron	6.25	349
Zinc	6.25	409
Total	13.5	758
Copper-Nickel-Zinc Unit:		
Copper	4.38	265
Nickel	4.38	244
Zinc	4.38	272
Total	13.5	781

The combination of copper, zinc and nickel gave similar results to the other metal combinations. Gas production was normal as long as sulfates were included with the daily feed. However, when the sulfates were removed and there was no means to precipitate the heavy metals as the insoluble sulfides, gas production rapidly decreased and total failure resulted.

The last graph shown in Figure 52 is the result of the iron-zinc combination. In this study it was reasoned that since iron alone was not detrimental even without sulfides, sulfides should not be required for the iron, but only for the zinc. Thus, only enough sulfides need be present to

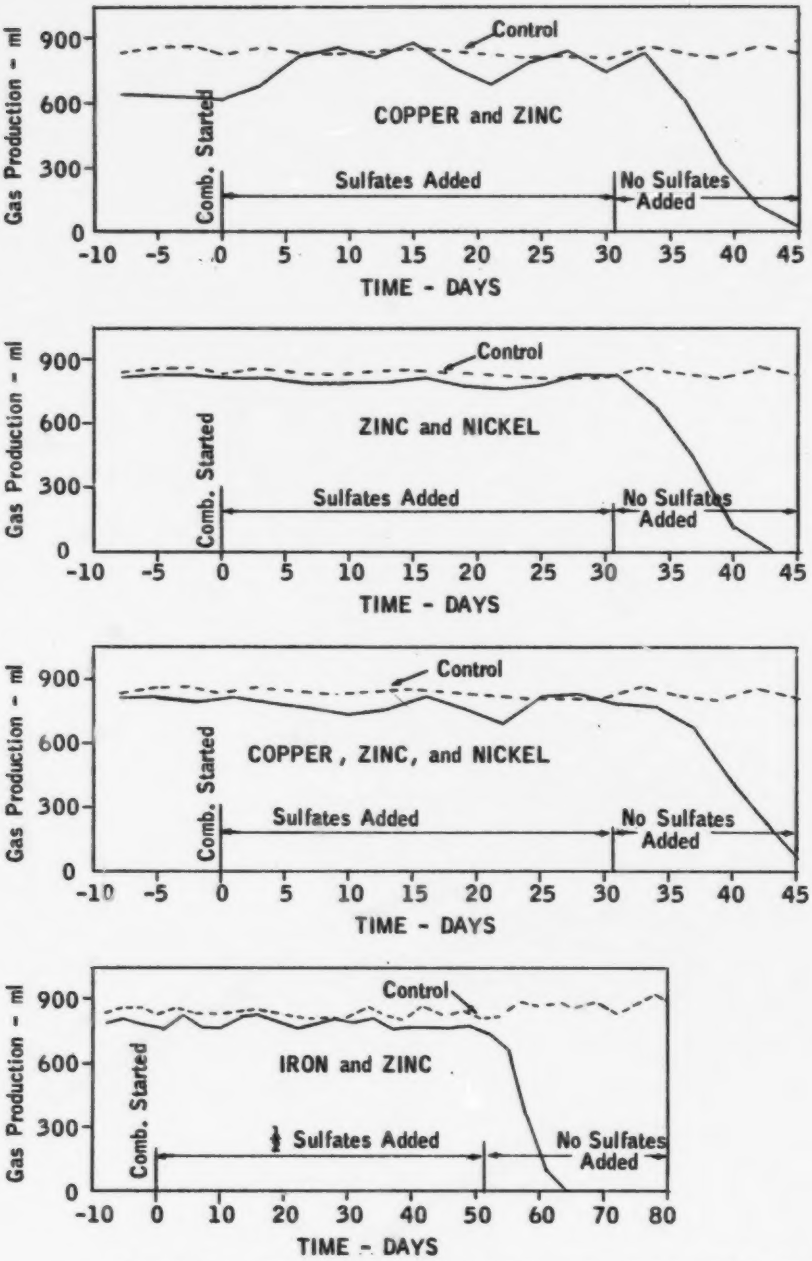


Figure 52 - Daily Gas Production for Digesters Receiving 13.5 mM Concentration of Combination of Heavy Metals

precipitate the added zinc. Table 17 indicates that zinc sulfide is much more insoluble than ferrous sulfide and so should precipitate first. To see if this would be the case, only zinc was added as the sulfate salt, while iron was added as the chloride salt.

From Figure 52 it can be seen that addition of only sufficient sulfate or sulfide precursor to precipitate the zinc was sufficient to maintain normal digester operation. Such operation was continued for 52 days. At that time, all sulfate additions were discontinued and then rapid digestion failure occurred.

This study has indicated that combinations of heavy metals are not toxic, providing sufficient sulfides are present to precipitate all the toxic, heavy metals. When the sulfide addition drops below this requirement, rapid failure of digestion occurs. It has also been shown that iron is not toxic when added with the daily feed, and that this metal does not need to be included in an evaluation of the total requirements for sulfides to prevent heavy metal toxicity. The toxic heavy metals, all of which are more insoluble than iron, can successfully compete with iron for the sulfides available. This is important in heavy metal toxicity control. The study also indicates that the concentration of each individual heavy metal is not the important parameter. To determine the toxicity of heavy metals, it is required that the sum total of the equivalent concentration of all be determined.

Figure 53 shows the gas production and volatile acids concentration which resulted when sulfates were removed from the copper-zinc digester, as well as the zinc-nickel digester. Again, it can be seen that gas production decreased rapidly before volatile acid concentration increased significantly. Complete failure also occurred before the volatile acid concentration reached 1,000 mg/l. It is also interesting to note that again, in the digester receiving nickel, the gas production was almost zero before the volatile acid concentration increased at all. Again, when complete failure was apparent, the volatile acid concentration had only reached about 300 mg/l. It thus appears that nickel is extremely toxic to the organisms producing volatile acids and that with this metal complete failure could occur with practically no indication of this failure being given by the volatile acid concentration. The change in volatile acids is thus a relatively poor parameter by itself to indicate the onset of heavy metal failure of a digester.

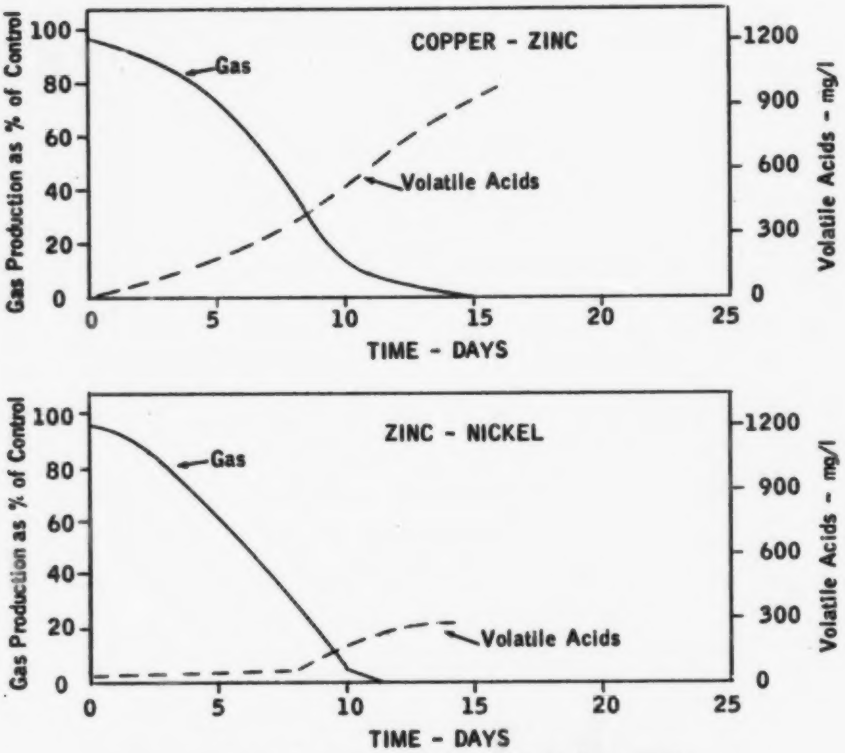


Figure 53 - Gas Production and Volatile Acid Concentration for Digesters Receiving Combinations of Metals as Shown for Time Period Beginning at Termination of Sulfate Addition

Table 21 shows the changes in gas analysis in two of the combination digesters which occurred during failure. This represents the changes which occurred after sulfates were removed from the daily feed. It can be seen that the percentage of methane in the gas remained practically the same throughout the digestion failure process.

TABLE 21

EFFECT OF TOXICITY FROM COMBINATION OF HEAVY METALS ON METHANE FRACTION OF GAS

Heavy Metal Combination	Days After Change to Chloride Salt	Gas Production in % of Control	% Methane in Gas
Copper-Zinc	1	91	65
	8	39	64
Zinc-Nickel	1	91	64
	8	29	66

For these combinations as well as for the individual nickel failure change in gas composition could not have been used as an indicator of impending failure. Thus, two of the common parameters used as indicators of impending failure would have been of little value here.

Measured Sulfide Concentrations

The sulfide concentrations measured in the various digesters during operation is of interest to indicate the extent of sulfate reduction, as well as to show the possible recoveries of sulfides obtained from the various heavy metal precipitates. Figure 54 indicates the concentrations of total sulfides, soluble sulfides, and sulfides in the gas in the digesters fed individual metal sulfates at a concentration of 400 mg/l as equivalent sulfur. The total sulfide concentration in the digesters receiving zinc and iron was close to 400 mg/l, corresponding to the quantity of sulfur introduced to the digesters. The concentrations started out low when introduction of the heavy metals and sulfates were first initiated and reached close to equilibrium concentrations after about 40 to 60 days of operation.

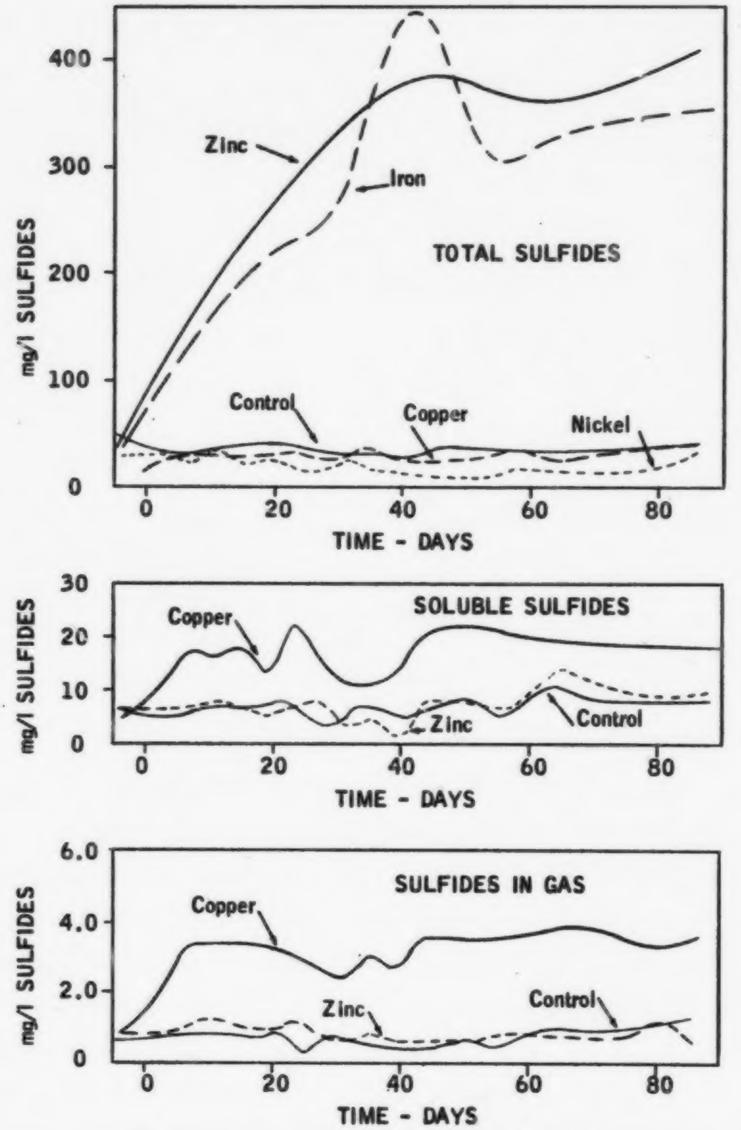


Figure 54 - Various Forms of Sulfides in Digesters Receiving 400 mg/l Sulfate Sulfur with Individual Metal Salts

The measured total sulfide concentration for the copper and nickel units, however, never exceeded that of the control, since the sulfides of copper and nickel are non-acid liable and are not measured in the standard sulfide test. Thus, although the sulfates were reduced to sulfides in these units, the normal test for sulfides will not indicate this. The sulfide recovery with the zinc sulfide was close to 100 percent, while that for iron was slightly below this. Between days 30 and 55, the total sulfide concentration in the iron unit took an erratic increase and then decreased back to normal. However, an adequate explanation for this unusual rise could not be given.

The graph for soluble sulfides indicates a peculiarity with copper. All the metal containing units except copper had a soluble sulfide concentration similar to that of the control. The soluble sulfide concentration for the zinc unit is shown in Figure 54 to indicate this. However, the copper unit had a much higher soluble sulfide concentration. It is possible that the cupric copper was reduced to cuprous copper, in the digester, which would stoichemetrically require less sulfides for precipitation. Thus, the additional sulfides would be released as soluble sulfide as shown. The slight decrease in gas production with higher copper concentrations shown in Figure 50, is perhaps a result of just such a change. This is speculation, and no additional data was obtained to verify these points.

The last graph in Figure 54 indicates sulfide concentration in the gas. The high concentration of sulfide in the gas in the copper unit is what would be expected, due to the higher soluble sulfide concentration as indicated above. The sulfides in the gas in all other metal containing units were similar to that of the control as indicated in the graph for zinc.

Effect of High Iron Concentration

Since ferrous iron was found to be non-toxic to digestion, even in the absence of sulfides, it was desired to determine if there was a practical upper limit to the quantity of iron which could be added. In this study, a ferric compound rather than a ferrous compound was used. Initially, a ferric chloride solution was added along with the daily feed to give a concentration of 698 mg/l iron. This addition was continued for 72 days. As shown in Figure 55, the gas production was only slightly affected by this concentration.

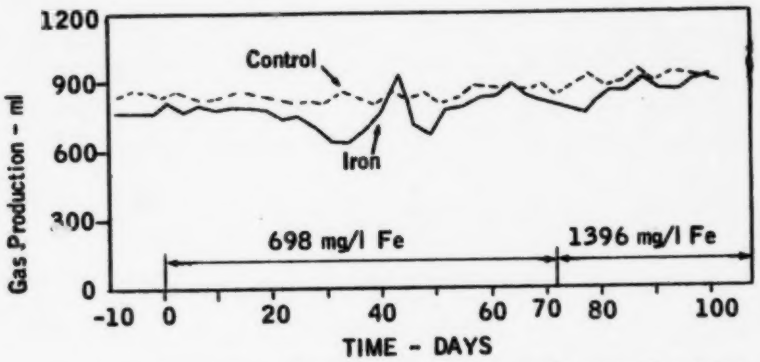


Figure 55 - Daily Gas Production for Digester Receiving Iron Fed as $FeCl_3$

After day 72, the concentration of ferric chloride was doubled to give a concentration of iron in the feed of 1,296 mg/l. This addition was continued for 32 days. Figure 55 indicates that during this time, gas production was affected only slightly. Other digestion parameters were normal during this time. It thus appears from this study that very high concentrations of iron can be tolerated in anaerobic digestion, providing the iron is added continuously on a daily feed basis, rather than being added on a slug basis.

The tolerance of anaerobic digestion for iron is probably related to the fact that (1) iron is not very toxic, and (2) precipitation of iron as the hydroxide by hydrolysis is sufficient at normal digester pH ranges, so that only a low, non-toxic concentration of iron will remain in solution. One point that should be noted is that during hydrolysis of iron, the pH in the digesters decreased. Such decrease in pH had to be controlled by addition of more buffering materials.

D. Discussion

The results of these studies indicate the significant role played by sulfides in the reduction of heavy metal toxicity. Sulfides are formed during digestion from protein degradation and sulfate reduction, and precipitate the heavy metals, rendering them non-toxic. The quantity of sulfides and sulfide precursors varies widely for different sludges and this, no doubt, is the reason for the wide range in toxicity found for the heavy metals in practice.

Another significant point from this study is that the sum total concentration of the heavy metals present is of importance, rather than the concentration of each individual metal. Toxicity will result only when the sum total concentration of the toxic heavy metals exceeds the equivalent concentration of sulfides present for precipitation. Thus, any study of possible heavy metal toxicity must include determinations of all heavy metals, as well as the total concentration of sulfides available for precipitation. However, such a study need not include iron as one of the toxic heavy metals. Fortunately, iron is one of the most soluble of the heavy metal sulfides, as well as being non-toxic. Thus, the sulfides formed during digestion would be available first for precipitation of the toxic heavy metals and only then would iron be precipitated. This is an important consideration.

Previous studies have for the most part reported heavy metal concentrations in percent of either the total or volatile solids fraction of the digested sludge. Such a procedure of reporting may have some validity in that the sulfides formed for precipitation of heavy metals are perhaps somewhat related to the volatile solids concentration. This, however, assumes that the major precursors of sulfides are insoluble protein materials. However, total solids analyses give a poor indication of the quantity of sulfates which might also be present and available as precursors of sulfides for heavy metal precipitation.

TABLE 22

CONCENTRATION OF HEAVY METALS IN DIGESTERS 70 DAYS AFTER BEGINNING OF METAL ADDITIONS

		Digested Sludge Concentration		Metal Concentration in Digest		
Metal Added	Metal Conc. in Feed mg/l	Total Solids - %	Volatile Solids % of Total	mg/l	% of Total Solids	% of Volatil Solids
Control	0	0.828	57.4	--	--	--
Copper	794	1.02	57.1	772	7.7	13.5
Zinc	817	0.904	51.3	795	8.8	17.2
Nickel	734	0.871	53.7	713	8.2	15.3
Iron	698	0.815	53.0	679	8.35	15.7

At the time indicated in this table, digestion was proceeding normally. It can be seen that the heavy metal concentrations were well over 10 percent of the volatile solids concentration, which is much higher than any previous study noted. At such exceptionally high concentrations, the digesters operated well as the heavy metals were precipitated by sulfides formed from sulfate reduction. Thus, the fallacy in basing allowable heavy metal concentrations on the dried solids concentration can readily be seen.

An alternate study to evaluate the toxicity of copper and zinc to anaerobic digestion was conducted using the Warburg apparatus and mixed liquor seed from digesters receiving pure acetic acid waste as described in Section II of this report. Under these controlled conditions, the sulfide concentration in the digesters was kept very low, normally in the range of 5 to 8 mg/l.

Thus, the potential of these systems for precipitation of heavy metals was also low. These studies indicated that copper and zinc concentration as low as 10 mg/l resulted in the complete cessation of gas production. However, when sufficient additional sulfides were added to precipitate the heavy metals, slug additions of heavy metals up to at least 100 mg/l could be tolerated with relatively little detrimental effect on gas production. Increase in pH by addition of alkalinity or removal of carbon dioxide to decrease the hydrolysis solubility of the heavy metals proved of no benefit. Thus, these studies give additional support to the important role of sulfides in reducing heavy metal toxicity, even at relatively low heavy metal concentrations.

This study has supported the findings of Masselli and Masselli (48) which indicated that the toxicity of the heavy metals can be prevented by addition of sufficient sulfides for precipitation. Thus, a relatively easy procedure is available for heavy metal control. If heavy metals are the cause of digester upset, the addition of an excess of sulfides will relieve the problem. Such an addition can also be made if heavy metal toxicity is simply suspected, and if the inhibition is then relieved, the suspicions would be fairly well confirmed.

The quantity of sulfide required is approximately 1/2 mg/l for each mg/l of the toxic heavy metal present. Since sulfides are quite corrosive and also toxic themselves, excessive concentrations should be prevented. Such problems may also be minimized by adding an iron-containing compound along with the sulfides. This would serve to hold the sulfides in to allow more complete reaction with the heavy metals.

One additional control procedure which could be followed if heavy metals occasionally reach a digester is to add a sulfide precursor to the digester. Thus, ferrous sulfate could be added to build up the total sulfide concentration in the digester, the iron serving to hold the sulfides rather than allowing them to escape as the gas. Such a procedure could also prevent an excessive concentration of soluble sulfides, which also might upset digester operation.

This study has shown that the toxic effects of heavy metals can be relieved by precipitation with sulfides. The previous studies reported in this report indicated also that soluble sulfides, by themselves, are toxic. However, when sulfides and heavy metals interact together, they form a co-precipitate which relieves the toxicity of both materials. Thus, the principle of toxicity removal by precipitation can be mutually applied to both materials.

E. Conclusions

1. Copper, zinc, and nickel heavy metal salts are toxic to anaerobic digestion.

2. Exceptionally high concentrations of iron can be tolerated in anaerobic digestion, if added daily along with the daily feed.

3. The toxic heavy metals are precipitated and rendered non-toxic in the presence of an equivalent concentration of sulfides.

4. The total concentration of toxic heavy metals which can be tolerated is directly related to the quantity of sulfides present for precipitation.

5. The sum total concentration of heavy metals and the availability of sulfides to precipitate this total concentration must be considered in evaluating heavy metal toxicity.

6. The addition of sulfides or sulfide precursors to relieve heavy metal toxicity is a sound control procedure.

7. The addition of sulfide or sulfide precursors has allowed the presence of much higher than 10 percent of heavy metals on a dried volatile solids basis without inhibiting the digestion process.

V. VOLATILE ACID EFFECTS

A. Previous Studies

Acetic, propionic, and butyric acids are the major volatile acids formed as intermediates in the anaerobic treatment of complex organic materials. The majority of the methane formed in this process results from the decomposition of these acids by a group of bacteria called "the methane formers."(39) A sudden increase in volatile acid concentration indicates the methane formers are not removing the acids as quickly as they are formed, and this is one of the first indications of unbalanced digestion. Although it is generally agreed high volatile acids and unbalanced digestion go together, it is still actively debated whether a high volatile acid concentration is the result of imbalance or the cause.

Although the volatile acids serve as the major source of "food" for the methane bacteria, Buswell and coworkers(40) in 1936 suggested that a high concentration of volatile acids was inhibitory to the digestion process, even though a neutral pH be maintained. McCarty and McKinney(41) recently have shown, however, that acetic acid, the most prevalent acid formed in methane fermentation(39) was not toxic to the methane bacteria even at concentrations of 10,000 mg/l or more. Since then, Buswell and Morgan(42) indicated that perhaps some volatile acids are more inhibitory to the methane bacteria than others. They proposed propionic acid is detrimental to digestion, based partly on the reports of Wachsman and Storck(43) on the lysis of protoplasts by propionic acid. They also indicated that propionic acid was found to be present in several digesters which had failed.

Buswell and Morgan(42) thus suggest high volatile acids may be toxic to the "methane formers" and hence the cause of "stuck digesters." However, there is considerable evidence to indicate the volatile acids are not the cause, but the result of unbalanced conditions and as such are not harmful to the methane organisms. In this case, proper digester control would require that the initial cause of the upset be found and corrected. Thus, proper digestion could not be obtained simply by reducing the volatile acid concentration. This study was instigated to provide more evidence to prove or disprove these theories. Here the effect of high concentration of the volatile acids, acetic, propionic, and butyric, both individually and in combination, on the digestion of sewage sludge was investigated.

B. Experimental Procedure

In this study laboratory digesters were operated to simulate field conditions of operation. The digesters were initially seeded with digested sludge from a municipal treatment plant. Additions of raw sludge and removals of digested sludge from the digesters were then made daily. After satisfactory digestion was accomplished, a high concentration of volatile acid salts was suddenly added along with the daily raw sludge feed. This slug feed of neutralized volatile acids simulated a sudden increase in volatile acids concentration resulting from temporarily unbalanced digestion conditions and subsequent neutralization with lime or other alkali to prevent a pH drop and a "stuck" digester. After the volatile acids were added, the digesters were operated as normal with daily additions and withdrawals of sludge. The effects of the sudden increase in volatile acid concentration on digester performance were then observed.

The digesters used consisted of a series of 2-liter bottles filled to the 1-1/2 liter mark with sludge as illustrated previously in Figure 2. Each digester was connected to a gas collection tube containing an acidified saturated salt solution. Sludge additions and withdrawals were made anaerobically through tubes connected through the top of the digester. Prior to and after sludge additions and withdrawals, the digesters were thoroughly mixed so composite samples could be obtained. The digesters were operated in a room with a constant temperature of 35 ± 0.5°C. The digested sludge seed and raw sludge feed for digestion were homogenized in a Waring blender for 15 seconds. This helped eliminate large particles which might tend to clog the digester tubing.

Routine analyses conducted to evaluate digester performance consisted of total and volatile solids, pH, alkalinity, gas production and composition, and volatile acid concentration. Separation and evaluation of individual volatile acids in the digesters was performed by the paper chromatography procedure reported previously.(44) In the first study reported below, 100 ml of raw sludge was added daily, resulting in a theoretical detention time in the digesters of 15 days. In all subsequent studies, only 75 ml of feed was used, resulting in a detention time of 20 days.

C. Results

Effect of Mixed Volatile Acids

The effect of suddenly increasing the volatile acid concentration to 6,000 mg/l as acetic acid by adding 2,000 mg/l each of acetic, propionic and butyric acids was first evaluated. Raw primary sludge obtained from the Nut Island Treatment Plant, Boston, Massachusetts was used for this study. This sludge was diluted with an equal mixture of raw sludge and tap water, and blended to make a thin and homogeneous mixture. Four digesters were operated in a 15-day detention time. In three of these digesters the alkalinity was increased by daily additions of sodium bicarbonate. By slowly increasing the alkalinity in these digesters, less alkalinity had to be added at the time of the slug volatile acid addition to maintain the proper pH. Thus, the effect of any "salt toxicity" as reported by McCarty and McKinney (41) could be evaluated along with the effect of the mixed volatile acids. The alkalinity of the individual digesters prior to the volatile acids addition are shown in Table 23 and in Figure 56. While building the alkalinity up to 9,800 mg/l in the one digester, the effect of "salt toxicity" was noted. This resulted when the alkalinity had reached 9,000 mg/l and was evidenced by a marked decrease in gas production and increase in volatile acids. After a period of about 10 days, however, the bacteria became acclimated to the high salt content, and the digester returned to normal operation prior to the addition of the volatile acid salts. The portion of the volatile acids which were neutralized with sodium hydroxide prior to the acid additions are also shown in Table 23.

Table 23

Digester Alkalinity and Fraction of Mixed Acids Neutralized Prior to Additions

Initial Digester Alkalinity mg/l	Volatile Acids Added - mg/l as Acetic Acid	
	Neutralized	Unneutralized
2600	6000	0
4400	4000	2000
6000	2000	4000
9800	0	6000

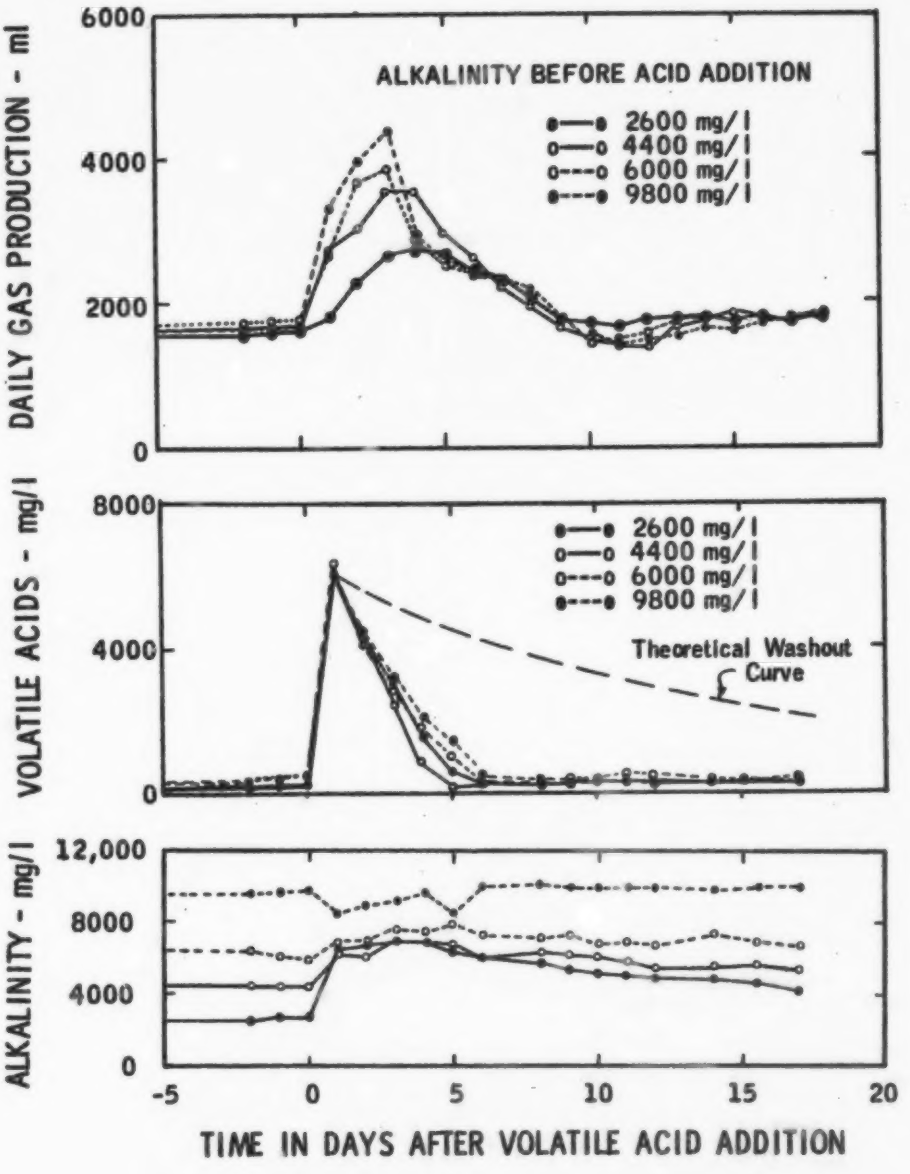


FIGURE 56-EFFECT OF SUDDEN ADDITION OF 6000 mg/l OF MIXED VOLATILE ACIDS ON DIGESTER PERFORMANCE

Figure 56 shows the gas production, volatile acids concentration and alkalinity in the individual digesters before and after acids addition. The different quantities of gas evolved in the different digesters is the result of the CO_2 production from the neutralization of the free volatile acids added to the different digesters by the bicarbonate alkalinity. The digester with an initial alkalinity of 2,600 mg/l received only neutralized acids, and so the increase in gas production represents the stimulation to digestion which resulted from addition of the acids. Although the carbon dioxide production differed in all the digesters as indicated above, gas analysis showed that the quantity of methane produced was the same in all digesters. In all cases the rate of methane production was greater after acid addition than before.

The volatile acids thus did not inhibit, but rather stimulated digestion. The volatile acids added were rapidly utilized as shown in Figure 56. Within one week they were completely destroyed and digester operation had returned to normal.

The theoretical washout curve for volatile acids shown in Figure 56 indicates how the added acids would have decreased in concentration if they were removed only by the dilutional effect of the daily additions and removal of sludge from the digesters. The rapid decrease of all the acids below the theoretical curve is indicative of their rapid rate of utilization. The decrease in volatile acids in all digesters was approximately the same, indicating that at this level it made little difference whether the alkalinity was in the digester prior to acid addition or was added subsequent to an increase in volatile acids. The stimulation to digester performance which was obtained also indicates there was no sodium salt toxicity present.

Effect of Individual Volatile Acids

In this run 6,000 mg/l as acetic acid of each individual acid was added to a different digester. The concentration of solids in the daily feed was reduced over the previous run so that the activity of the methane formers in the digester would be somewhat lower than in the previous study. It was felt that this would result in less rapid utilization of the volatile acids, allowing them to remain for a longer period at high concentration to increase the chance of a possible inhibitory effect. Also, the digestion time was increased to 20 days to reduce the quantity of acids washed out by the daily feeding and withdrawals. A different raw sludge was also used. This was a mixed primary and trickling filter sludge obtained from the Palo Alto, California, sewage treatment plant. The sludge was diluted with an equal part of tap water and blended as before. The normal sludge analysis and digester performance prior to acid additions for the remainder of the studies reported are indicated in Table 24.

Table 24

DIGESTER PERFORMANCE PRIOR TO ACID ADDITIONS

Raw Sludge - 1.16% total solids
Detention Time - 20 days
Volatile Solids Loading - 0.028 lb/cu.ft/day
Volatile Solids Reduction - 59%
pH - 6.7
Alkalinity - 1500 mg/l
Gas Production - 0.3 cu.ft/cu.ft

Four digesters were used, one serving as a control, and the other three each receiving 6,000 mg/l of the individual volatile acids neutralized to pH 7.0 with lime. The gas production which resulted after the addition of the acids is shown in Figure 57 and the individual volatile acid concentrations in the three digesters to which volatile acids were added are showing in Figure 58. The gas production in the control varied markedly from day to day which reflects the varying concentration of the raw sludge feed. Throughout this run, the pH in the digesters did not vary outside the limits of 6.7 to 7.2, and the methane in the gas remained between 64 and 74% in all cases.

The digesters to which acetic and butyric acids were added responded as in the previous run. The gas production increased significantly over the control and the excess volatile acids were rapidly utilized. The rate of utilization, however, was somewhat less than in the preceding run partly because of the lower activity of the methane bacteria present.

The propionic acid, however, appeared to have a somewhat inhibitory effect on gas production. Gas production decreased below that of the control for about one week, after which it began to rise, and by the second week had exceeded that of the control. Thus, there appeared to be an initial inhibitory effect to which the bacteria in a short time could acclimate. The volatile

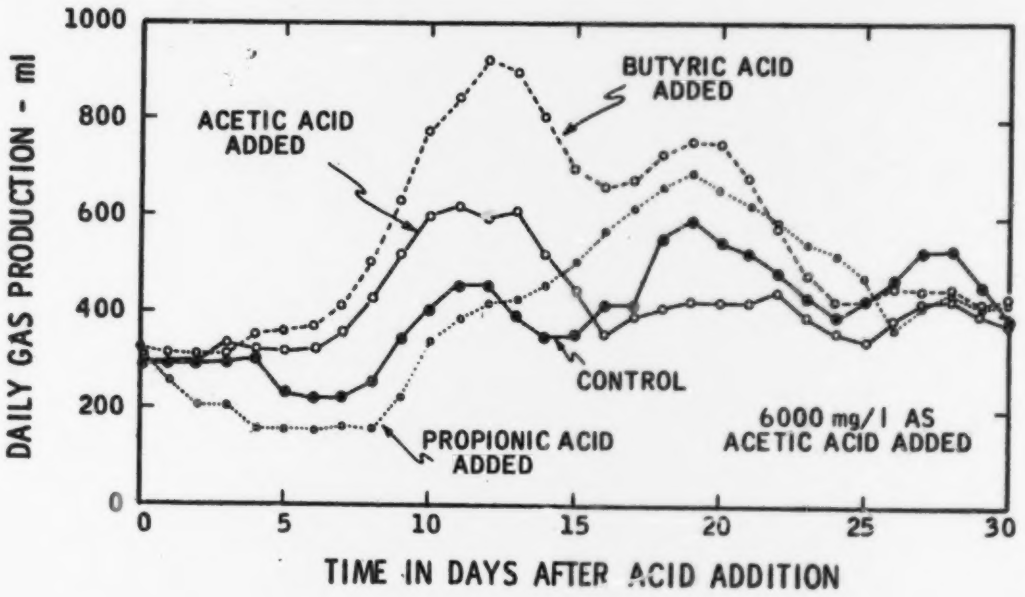


FIGURE 57- EFFECT OF INDIVIDUAL VOLATILE ACIDS ON GAS PRODUCTION

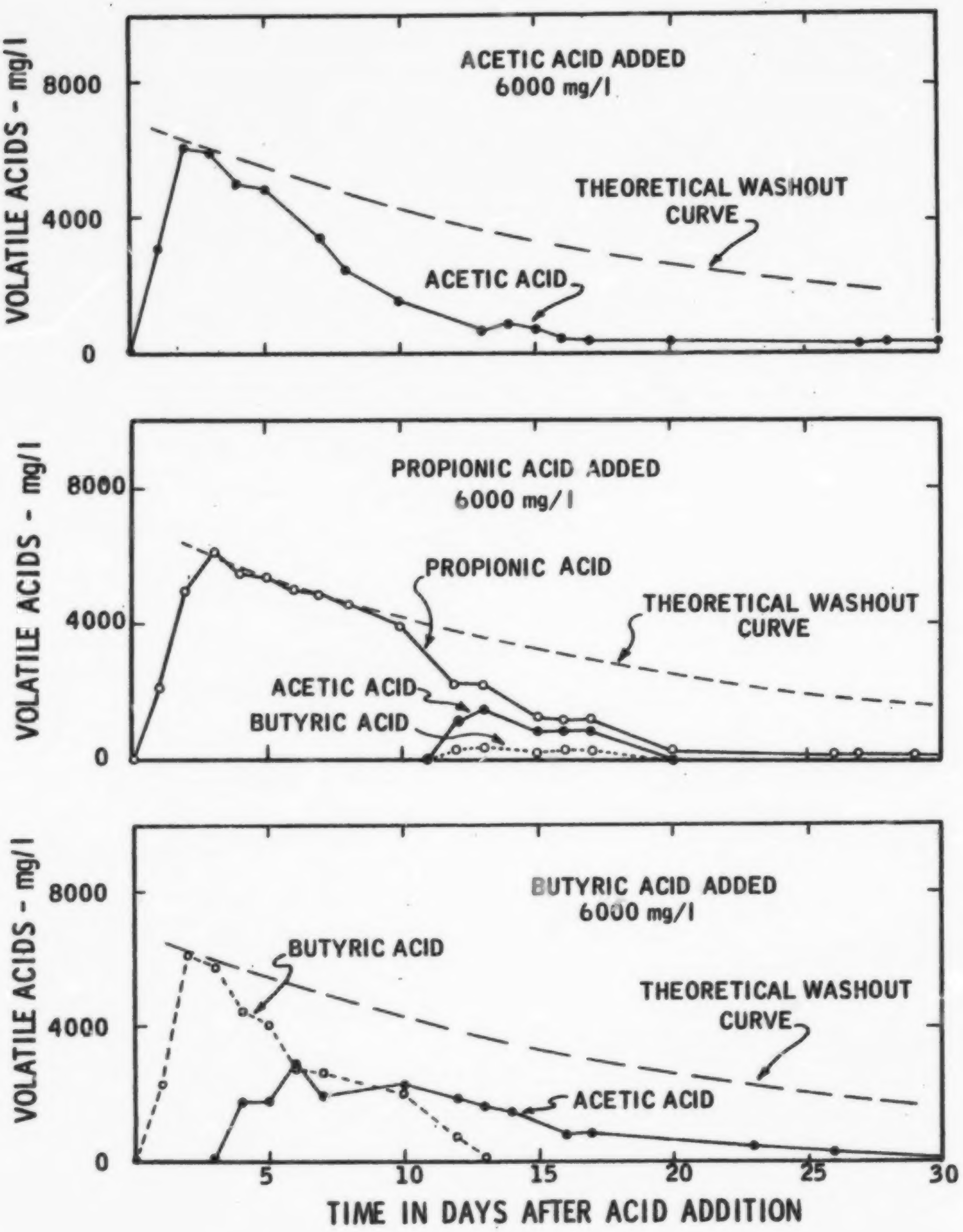


FIGURE 58- VOLATILE ACIDS PRESENT IN DIGESTERS TO WHICH INDIVIDUAL VOLATILE ACIDS WERE ADDED

acids analysis indicated that during this period no additional volatile acids were produced and the propionic acid concentration decreased along the theoretical washout curve. At the end of the week period when the gas production began to increase, the propionic acid concentration decreased below the curve. Because there was no additional increase in volatile acids above that added, it appeared the inhibitory effect was perhaps harmful to the organisms producing the volatile acids, rather than to those responsible for volatile acid decomposition.

Effect of Different Propionic Acid Concentrations

In this run the effect of a sudden increase to different concentrations of propionic acid on digestion was studied. Here, four individual digesters were used and were fed mixed primary and trickling filter sludge as in the previous run. When operation was normal and the same in the 4 digesters, concentrations of propionic acid of 3,000 mg/l, 6,000 mg/l, and 8,000 mg/l as acetic acid were suddenly added to 3 of the digesters. Prior to these additions, the propionic acid was neutralized to pH 7.0 with lime. The 4th digester served as a control and received only the normal sludge feed. The effect of these additions on gas production is shown in Figure 59. Also the measured concentrations of individual volatile acids in the digesters after these additions are shown in Figure 60. As in the previous run, the pH remained between 6.7 and 7.2 and the methane content of the gas between 64 and 74 percent in all digesters throughout the study.

In all cases the sudden increase in propionic acid resulted in a decrease in gas production as in the previous run. The initial effect on gas production was similar for all 3 concentrations. However, in the digesters to which 3,000 mg/l was added, the gas production soon returned to normal, and then exceeded that of the control. During the period of increased gas production, the propionic acid was being rapidly utilized as shown in Figure 60.

With the two highest concentrations of propionic acid, the gas production remained below that of the control for about a week, and then began to increase rapdily as in the previous study. The gas production then soon exceeded the control while the propionic acid was rapidly being utilized as shown in Figure 60.

This run confirmed that a sudden increase in the concentration of propionic acid initially hinders gas production. However, after a short time

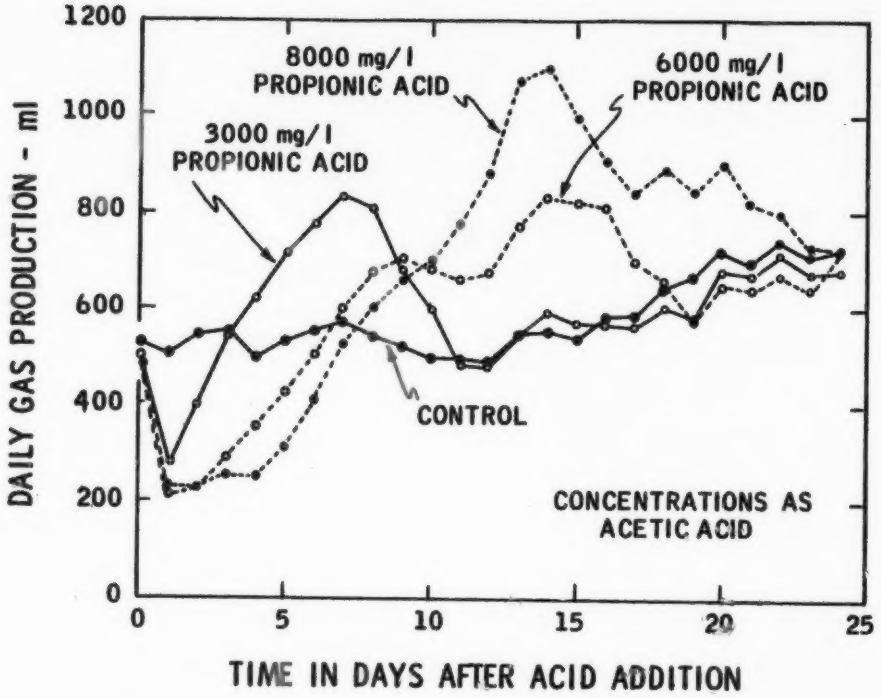


FIGURE 59-EFFECT OF PROPIONIC ACID ON GAS PRODUCTION

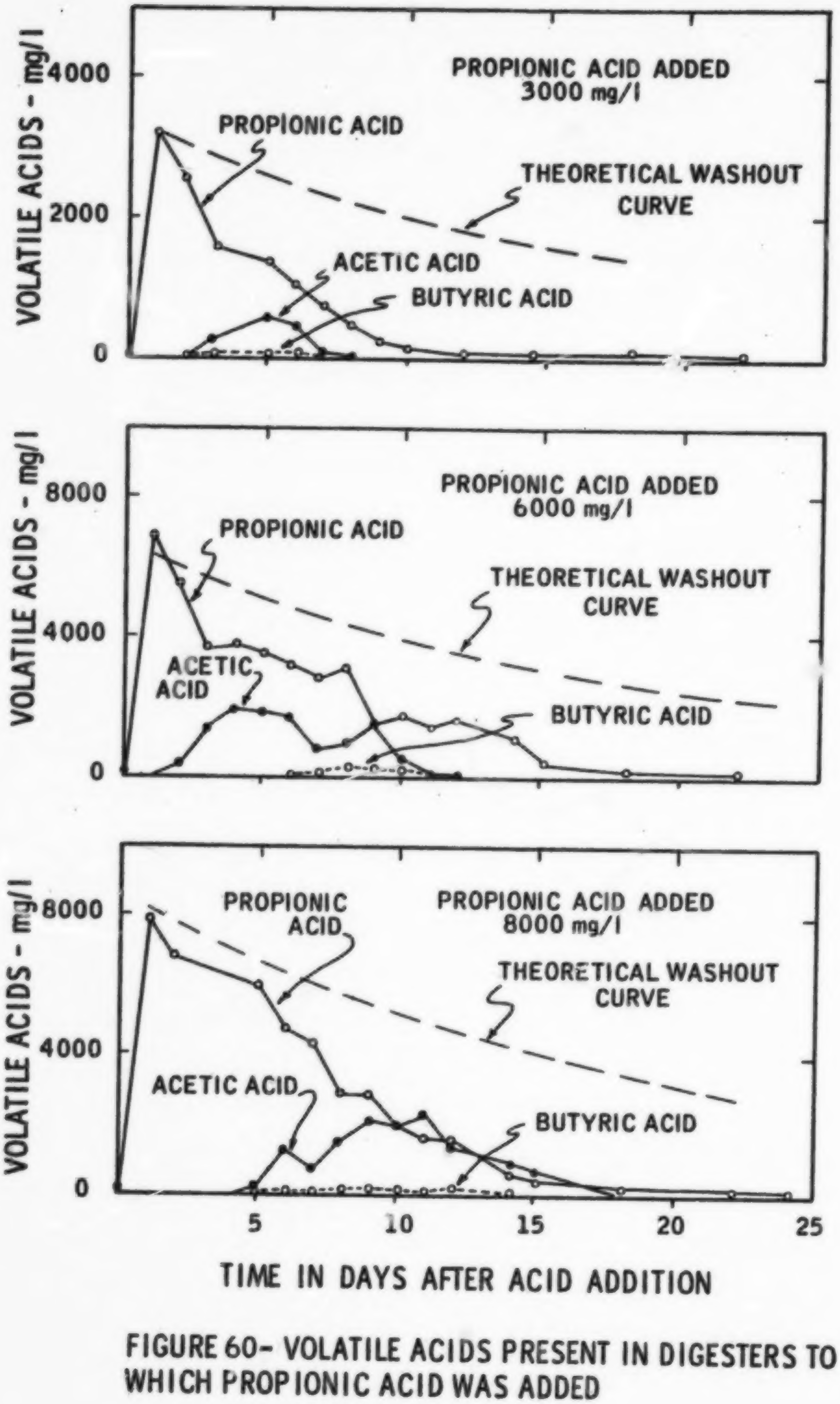


FIGURE 60- VOLATILE ACIDS PRESENT IN DIGESTERS TO WHICH PROPIONIC ACID WAS ADDED

the bacteria can acclimate to the inhibitory effect so that normal digestion can resume. Seldom would concentration of 6,000 to 8,000 mg/l of propionic acid be experienced even in severely "stuck" digesters. A concentration of 3,000 mg/l, however, might be expected and as found here, the period required for acclimation to this concentration is quite short.

Nature of Propionic Acid Inhibition

The previous two runs indicated that a sudden increase in propionic acid concentration could hinder gas production for a short period. A knowledge of the nature of this inhibition is desirable so that the true effect on digester performance can be evaluated. In Figure 61 is shown a schematic diagram illustrating the general pathways of anaerobic treatment of complex wastes. The wastes are first broken down by a miscellaneous group of facultative and anaerobic bacteria normally called the "acid-formers," to propionic and acetic acid by route A. A special group of methane bacteria then convert propionic acid to acetic acid by route B. The acetic acid formed both directly from the complex organics and also from propionic acid fermentation is then fermented to carbon dioxide and methane by another group of methane organisms through route C. If the bacteria responsible for fermentation by any one or combination of these three paths are affected by a sudden increase in propionic acid, then the gas production will decrease as observed in this study.

If only the organisms responsible for the fermentation of acetic acid by route C were affected, there would be a decrease in gas production also. However, a build-up of acetic acid would then result by way of paths A and B. Since a significant increase in acetic acid was not observed in this investigation, it appeared doubtful that the propionic acid inhibited this group alone.

Similarly, if the propionic acid affected only the methane organisms responsible for the route B conversion of propionic acid to acetic acid, gas production again would decrease. However, in this event there would be a significant increase in propionic acid concentration. Since this did not occur, this possibility appeared to be negative.

Two other possibilities may exist. For one, the sudden increase in propionic acid may have affected all routes in a similar fashion resulting in decreased gas production, without a resultant increase in any of the volatile acids. This might explain the result obtained. The other possibility is that

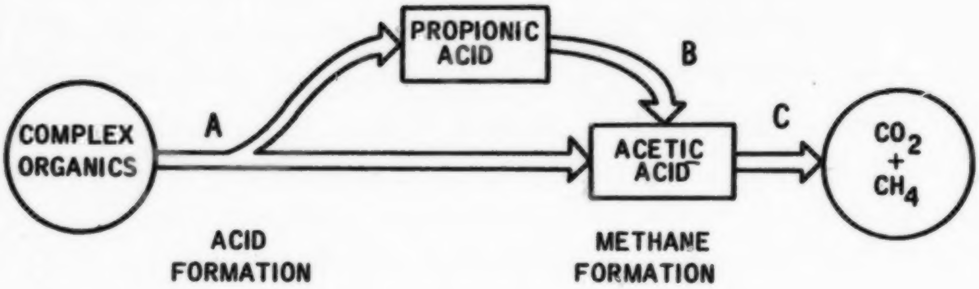

PROPIONIC ACID
B
COMPLEX ORGANICS
A
ACETIC ACID
C
CO_2 + CH_4
ACID FORMATION
METHANE FORMATION

FIGURE 61–GENERAL PATHWAYS OF ANAEROBIC TREATMENT OF COMPLEX WASTES

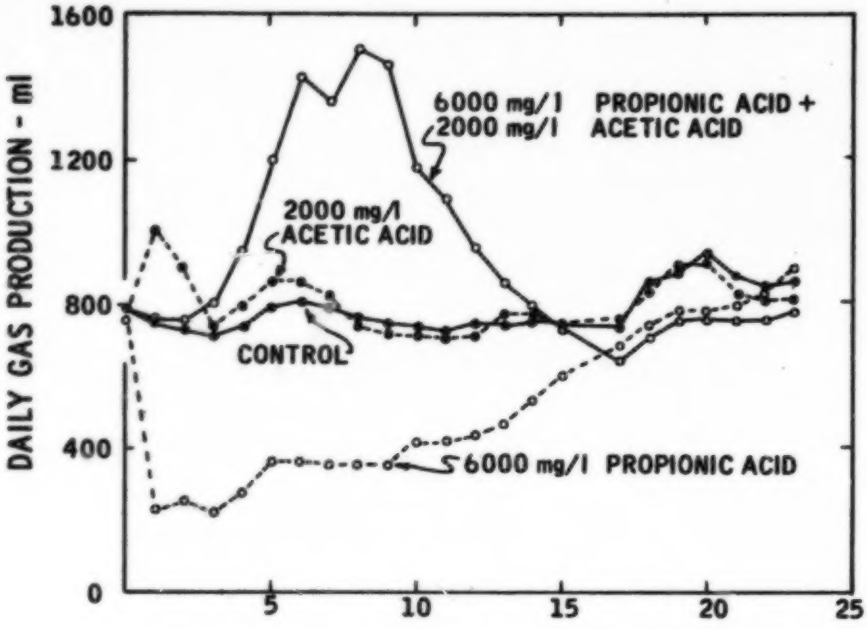
the "acid-formers" responsible for the conversion of the complex organics to propionic and acetic acid by route A, alone were affected. In this case there would also be a decrease in gas production without a resultant increase in volatile acid concentration.

Since both the latter two possibilities could explain the results obtained, it was desirable to conduct an additional study which might more strongly indicate one possibility over the other. To do this, four digesters were again operated at a 20-day detention time using the mixed primary and trickling filter sludge. When the digesters were operating normally, volatile acids were suddenly added to individual digesters in the following way.

The first digester received no volatile acids and served as a control. The second digester received 6,000 mg/l of propionic acid (as acetic acid) neutralized with lime. This digester as before should indicate the inhibitory effect of propionic acid. The third digester also received 6,000 mg/l of neutralized propionic acid, but in addition, 2,000 mg/l of neutralized acetic acid was added. The 4th digester received only 2,000 mg/l of the neutralized acetic acid.

If the propionic acid inhibited all the groups of bacteria responsible for anaerobic treatment, then digesters 2 and 3, both with 6,000 mg/l of propionic acid, should behave in a similar fashion with decreased gas production. On the other hand, if only the acid formers responsible for the conversion of complex organics to volatile acids were affected, then the sudden increase in volatile acids should significantly affect only digester 2, but not digester 3. This would be the case since in digester 3 the added acetic acid could then serve as a normal source of food for the methane organisms for the path C conversion.

This experiment should thus tell whether propionic acid inhibited only the acid formers, or inhibited both acid formers and acetic acid utilizing methane formers. The results are shown in Figures 62 and 63 showing gas production in the various digesters, as well as volatile acid concentrations. It can be seen that the propionic acid inhibited gas production only in digester 2, while digester 3 continued as well as, and then better than, the control. This run gives good evidence that a sudden increase in propionic acid concentration inhibits only the organisms responsible for the production of acetic acid, and not the methane formers responsible for the conversion of this



TIME IN DAYS AFTER ACID ADDITION

FIGURE 62–EFFECT OF PROPIONIC AND ACETIC ACID MIXTURE ON GAS PRODUCTION

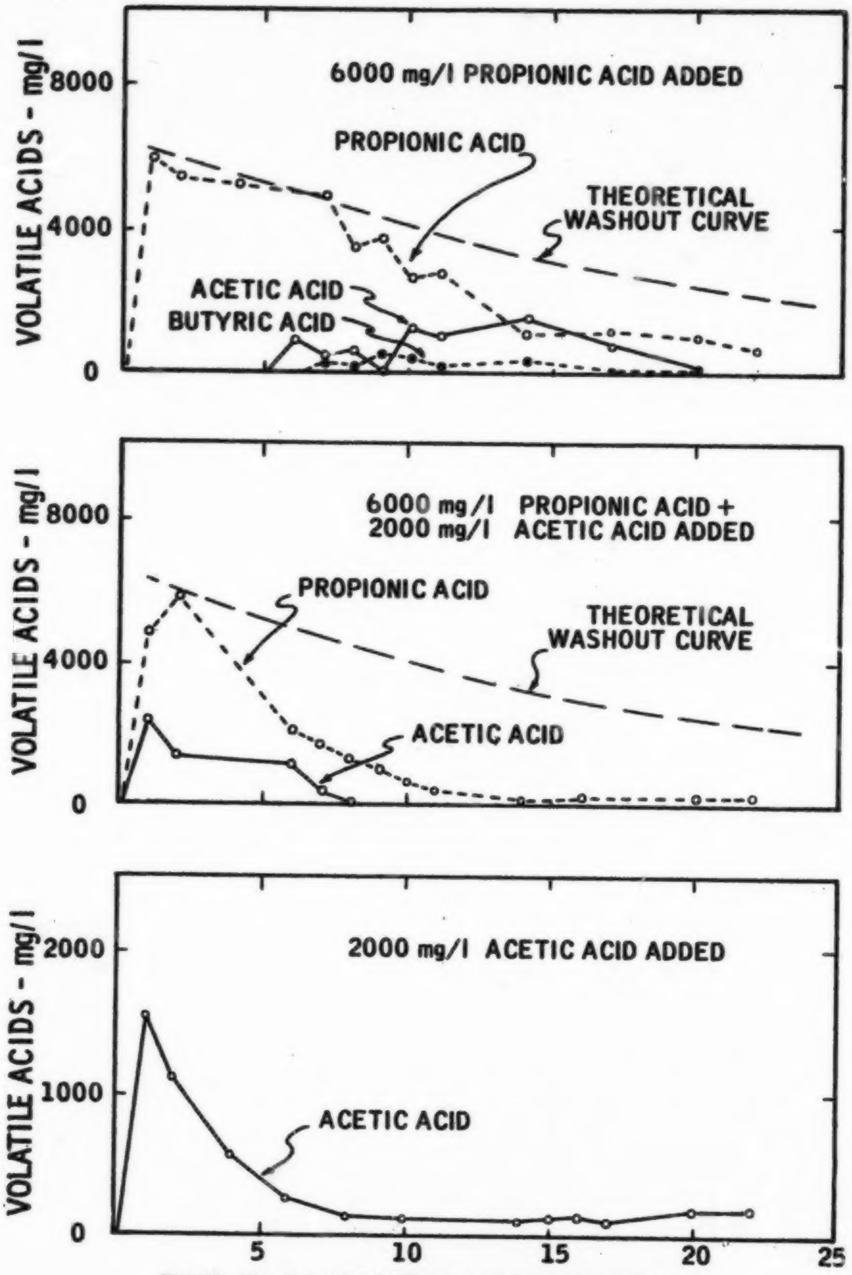


FIGURE 63-VOLATILE ACIDS PRESENT IN DIGESTERS TO WHICH MIXTURES OF PROPIONIC AND ACETIC ACID WERE ADDED

acid to methane and carbon dioxide. In the digester to which acetic plus propionic acids were added, the propionic acid was utilized much more rapidly than when propionic acid was added alone. This might indicate acetic acid has some additional beneficial effect. However, the digester with propionic acid alone did not seem to respond quite as quickly as in previous runs, which might also explain the difference.

D. Discussion

Sudden increases in the concentration of either acetic or butyric acids were found stimulatory, rather than inhibitory to the anaerobic treatment process. However, a rapid increase in propionic acid concentration did initially inhibit gas production somewhat. In all cases, this inhibitory effect was overcome by a short period of acclimation. This was true even with a propionic acid concentration as high as 8,000 mg/l as acetic acid.

Although high concentrations of propionic acid initially inhibited gas production, this inhibition was not accompanied by an increase in volatile acids. Therefore, propionic acid appeared to affect the bacteria producing the acids, rather than the "methane forming" bacteria which degrade the acids. Without acid formation, the main source of food for the methane bacteria was stopped and, hence, methane production dropped significantly. However, by adding acetic acid along with a high concentration of propionic acid, the food supply was returned to normal and gas production was then not affected. This verified that propionic acid is not significantly inhibitory to the methane bacteria. Thus, high concentrations of acetic, butyric, or propionic acids do not affect the methane bacteria and so should not be considered the cause of digester imbalance.

All reports of which the authors are aware on high concentrations of volatile acids in digesters could be explained on the basis that a rapid increase in volatile acids is the result and not the cause of unfavorable conditions. Factors causing such unbalance may be classified as "temporary" or "permanent." "Temporary" unbalance may occur during digester start-up before the methane organisms grow to sufficient population, as shown by Cassel and Sawyer (45). Such unbalance may also result from increased organic load as indicated by the work of Poland and Bloodgood (46). Also, ineffective mixing of high rate digesters may result in "temporary" unbalance and volatile acids build-up, as shown by Sawyer and Buzzell (47). A sudden decrease in temperature will also have the same effect. All these causes result in a "temporary" increase in volatile acids. In each case, if sufficient time is allowed for the methane population to establish itself, the volatile acids will then decrease and normal digestion will be obtained. It is important during this period, however, to maintain a neutral pH, since it is well known the methane organisms are severely affected by pH values below 6.0.

"Permanent" unbalance may be caused by several factors and will also result in increased volatile acid concentration. Among these are well known effects from toxic materials such as the heavy metals, and cyanides. Many other potentially toxic organic materials such as pesticides, surfactants, and organic solvents may also affect digestion. Toxic concentrations of these materials will inhibit the methane organisms and result in increased volatile acid concentrations. However, in this case even pH control may be of no avail since this will not remove the cause of the "permanent" unbalance. Here, the inhibitory material must be removed or reduced below the toxic concentration by dilution of the digester contents. Extreme cases may require emptying the digester contents and beginning anew.

A sudden increase in volatile acids should thus be considered the result of unbalanced conditions. When this occurs, the first thing to do is practice pH control, for if the pH drops the methane organisms will surely be affected and a long period may be required for these organisms to become re-established. Then, the cause of the increase must be found. If the cause is "temporary" in nature, then by maintaining pH control, digestion conditions will return to normal as soon as the methane organisms can achieve sufficient population. If the cause is "permanent" in nature, then successful digestion can only be accomplished by removal of the toxic materials. Here, pH control alone will not restore digestion. The difference between "temporary" and "permanent" unbalance may explain why some have found pH control alone effective, while others have not.

E. Conclusions

1. A sudden increase in the concentration of acetic and butyric acids to 6,000 mg/l, both individually and in combination stimulate, rather than hinder, the anaerobic digestion process, providing a neutral pH is maintained.

2. A sudden increase in the concentration of propionic acid alone to levels of 3,000 to 8,000 mg/l hinders gas production for up to one or two weeks, after which time acclimation occurs and gas production returns to normal.

3. A sudden increase in propionic acid does not affect the major methane bacteria responsible for the fermentation of acetic acid, rather it appears to inhibit the "acid-forming" bacteria.

4. High concentration of acetic, propionic, and butyric acids are the result, and not the cause of digester unbalance.

5. "Temporary" unbalanced digesters can be corrected by maintaining pH control.

6. "Permanent" unbalance in digesters cannot be corrected by pH control, unless the initial cause is first removed.

V. MISCELLANEOUS STUDIES

The preceding sections covered studies on toxic and inhibitory effects of the common materials often suspected of upsetting digesters. In this section a few miscellaneous studies performed during the course of this project are reported. The materials considered here were investigated as sidelights to the major studies, and in most cases, only sufficient information was obtained to indicate the relative effects of the various materials.

Miscellaneous Inorganic Salts

In this study several miscellaneous sodium salts were added to individual digesters on a slug basis to determine the relative effects of different anions on anaerobic treatment. These salts were added to 3/4-liter anaerobic digesters, operated in a similar fashion to those shown in Figure 2 of this report. These digesters had been receiving 2 grams/l/day of an acetic acid synthetic substrate. After the digesters were operating well, various sodium salts at relatively high concentrations were added one day along with the daily acetic acid feed. The effect of such addition on gas production and other digester parameters was then observed. No additional feeding of acetic acid substrate was made subsequent to the slug salt additions.

Table 25 indicates the concentrations of the various salts which were added to these individual digesters. The concentrations shown represent those resulting in the digester after the salts were added. Concentrations of salts were at a sufficiently high level, but low enough so any sodium inhibition would be minimized. The anions studied were chloride, nitrates, sulfates and phosphates. Except in the case of phosphates, the sodium concentration in all units was maintained the same so that any cation effects would be equal. Thus, any noted decrease in gas production would be related to the effect of the individual anions and not the cations. The rate of gas production after the slug addition was found to be essentially the same in the chloride, the phosphate and the sulfate units. These rates were very close to that of a control, which received only the acetic acid itself. Thus, these particular anions, by themselves, are relatively non-toxic to anaerobic treatment.

TABLE 25

CONCENTRATION OF SALTS AFTER ADDITION TO INDIVIDUAL DIGESTERS IN SLUG FEED STUDIES

				Anion Concentration	
Digester	Salt Added	Molar Concentration	Sodium mg/1	Anion	mg/1
1	NaCl	0.11	2500	Cl	3900
2	$NaNO_3$	0.11	2500	NO_3	6820
3	$Na_2SO_4 \cdot 10H_2O$	0.055	2500	SO_4	5280
4	$NaH_2PO_4 \cdot H_2O$	0.035	805	PO_4	3330
	$Na_2HPO_4 \cdot 7H_2O$	0.075	3450	PO_4	7120
	Total	0.11	4255	PO_4	10,450

However, the unit receiving sodium nitrate had complete failure, and gas production was practically zero. Thus, the addition of nitrates stopped the methane fermentation. After a period of about two weeks, the units were analyzed for residual acetic acid concentration and it was determined then that the acetic acid concentration in this unit had been reduced from 2,000 mg/1 to 290 mg/1. Since no methane was associated with this decrease, it was concluded that the acetic acid had been used by facultative organisms, using the nitrates as hydrogen acceptors.

From this study it can be concluded that the chlorides, sulfates, and phosphates have relatively little effect on anaerobic treatment. It is the cations with which they are associated that give the major problem. It is interesting to note that sulfates at the high concentration used had relatively little effect. However, if these sulfates were present for sufficient time to become reduced to sulfides, they would no doubt have produced a sulfide toxicity as noted in Section III of this report.

Although nitrates were found to have a detrimental effect on methane fermentation at high concentration, it is unlikely that a waste containing such a high nitrate concentration would be added to an anaerobic treatment process. If such a waste were available, it could be continually fed to the digester so that the nitrates would be reduced by facultative bacteria as rapidly as they entered and no toxicity would develop. The toxicity is only related to a sudden high loading of the nitrates. Nitrates no doubt act in similar fashion to oxygen, which is also toxic to methane bacteria. The effect of nitrates on digestion and their rapid reduction have been noted by Buswell (64), Straus (65), and Fischer, Rudolfs, and Zeller (66).

Fatty Acids

Long chain fatty acids are known to be toxic to microorganisms. This no doubt is true also for anaerobic waste treatment. However, most of the fatty acids which are added to digesters are added as insoluble calcium salts. Therefore, they are effectively removed from the biological environment and so are prevented from causing an inhibitory effect.

However, the toxicity of soluble fatty acids to methane fermentation has been demonstrated in several studies conducted during this investigation. In several digesters, sodium oleate was used as a synthetic feed. Since little calcium was present in this feed, the oleate entered the digester and remained in the soluble sodium form. Sodium oleate is the major component of castile soap, and effects from this would be similar to effects from other soluble soaps. Throughout these studies, conducted with daily feeding of sodium oleate, it has been found that when the sodium oleate concentration reached some point between 500 and 1,000 mg/l that inhibition resulted. Thus, satisfactory operation required that the oleate concentration be kept below this value.

The inhibition resulting from high concentrations of sodium oleate were easily overcome by the addition of sufficient calcium to precipitate the oleate. The addition of calcium chloride to sodium oleate digesters results in rapid precipitation and completely relieved any toxicity from this fatty acid. In the presence of the calcium, concentrations of sodium oleate of 2,000 mg/l or higher could be tolerated with no adverse effects on digester operation. Thus, again, the principle of precipitation has worked well in relieving toxicity in anaerobic treatment.

In additional experiments, other organic materials at high concentration have been found also to inhibit methane fermentation. For example, methanol in a concentration of about 1,000 mg/l seriously affected the methane fermentation process. However, concentrations below this were rapidly utilized and caused no problems. Thus, in anaerobic treatment, the same principles apply as in the aerobic treatment of toxic wastes. Many organic wastes are inhibitory to microorganisms at some high concentration. However, at lower concentrations these waste are readily degradable. These wastes can be successfully treated by biological processes providing they are added continuously to the treatment units, and are thoroughly mixed so that toxic concentrations do not develop By continual addition and mixing, these wastes are utilized as rapidly as they are added, and the concentration in the biological treatment unit never reach toxic levels, in spite of the fact that the concentrations in the incoming wastes may be excessively high.

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